National Research
Council Canada

Conseil national
de recherches Canada

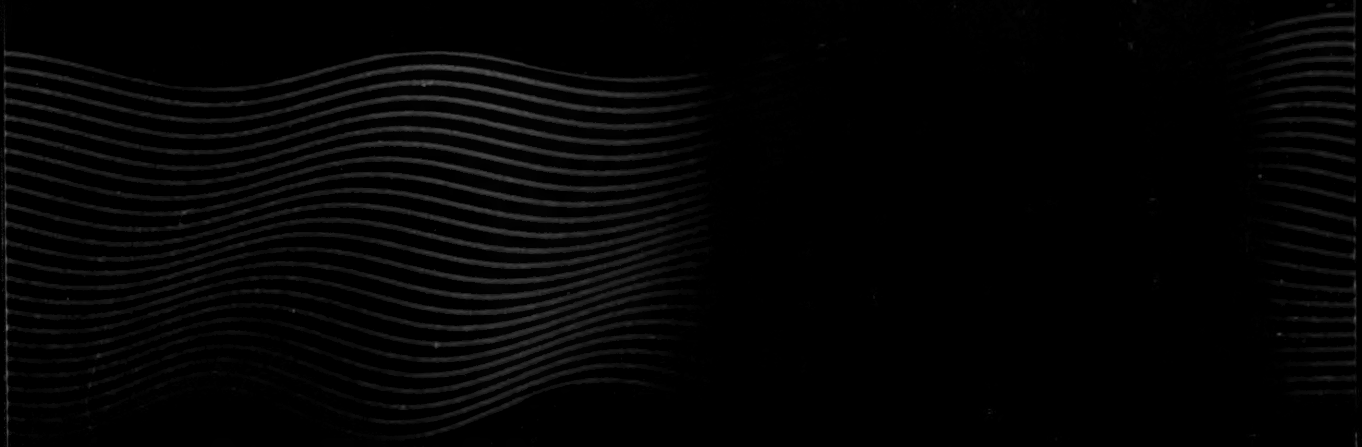
NRC · CNRC

Canadian Journal of Fisheries and Aquatic Sciences

Journal canadien des sciences halieutiques et aquatiques

100th Anniversary

100e anniversaire



http://www.nrc.ca/cisti/journals

ISSN 0706-652X / CJFSDX 58(9) 1703–1903 (2001)

The *Canadian Journal of Fisheries and Aquatic Sciences*, previously published as *Contributions to Canadian Biology* 1901–1925, *Contributions to Canadian Biology and Fisheries* 1926–1934, *Journal of the Biological Board of Canada* 1934–1937, and *Journal of the Fisheries Research Board of Canada* 1938–1979, is issued monthly to form annual volumes of 12 numbers plus supplements.

Editorial policy: The Journal publishes original research articles, perspectives (syntheses, critiques, re-evaluations), discussions (comment and reply), and rapid communications. Manuscripts may concern cells, organisms, populations, communities, ecosystems, or processes that affect aquatic systems. They may cover a range of disciplines including biology and ecology of marine and freshwater organisms, limnology, oceanography, physiology, toxicology, genetics, economics, disease, and management. Preference will be given to manuscripts that emphasize understanding of observed phenomena and interpretation of experimental results. The Journal encourages papers that lead from clearly stated purpose or rationale and from testable hypotheses, concepts, or questions to identifiable conclusions or syntheses. Such papers may amplify, modify, question, or redirect accumulated knowledge embodied in contemporary perceptions of a particular state of aquatic sciences. The Journal discourages papers that are essentially descriptive, except in emerging disciplines; have only site-specific or local applications (a certain year, place, taxon, chemical compound, etc.); only confirm previously established principles; or apply standard techniques without breaking new methodological ground.

Manuscripts for publication should be submitted to the Editors, *Canadian Journal of Fisheries and Aquatic Sciences*, Department of Zoology, University of Guelph, Guelph, ON N1G 2W1, Canada (519-824-4120 ext. 2646; fax: 519-767-9798; e-mail: cjfas@uoguelph.ca).

Submissions in either English or French are acceptable. The information must be original, that is, not copyrighted, published, or submitted elsewhere except in abstract form or unless by written consent of the Editor. NRC accepts no responsibility for statements or opinions expressed by a contributor. Acceptance of an advertisement, announcement, or other material does not imply endorsement by either the Journal's Editors or NRC. The use of proprietary names does not imply endorsement of the product or company.

Correspondence concerning accepted manuscripts and corrected proofs should be sent to NRC Research Press, National Research Council of Canada, Ottawa, ON K1A 0R6, Canada.

Instructions to Authors are published once a year in the first issue of each volume. Copies are available from NRC Research Press or at http://cjfas.nrc.ca. The **Publication Policy** of NRC Research Press is available at http://www.nrc.ca/cisti/journals/.

Microfilm: Issues from 1934 through the current volume can be purchased on 16- or 35-mm microfilm. Photocopies of individual articles or issues can be purchased from University Microfilms International, 330 North Zeeb Road, Ann Arbor, MI 48106, U.S.A.

U.S. periodical postage paid at Champlain, N.Y., and at additional mailing offices. Attention U.S. POSTMASTER: Send address changes to **Canadian Journal of Fisheries and Aquatic Sciences**, c/o USACAN Media Distribution Center, P.O. Box 2888, Plattsburg, NY 12901, U.S.A.

Publié sans interruption depuis 1901, le *Journal canadien des sciences halieutiques et aquatiques* a paru sous plusieurs titres : *Contributions to Canadian Biology* 1901–1925, *Contributions to Canadian Biology and Fisheries* 1926–1934, *Journal of the Biological Board of Canada* 1934–1937, et *Journal of the Fisheries Research Board of Canada* 1938–1979. Le Journal paraît tous les mois, formant ainsi un volume de 12 numéros, et offre en plus jusqu'à deux suppléments par année.

Politique éditoriale : On publie, dans le Journal, des articles rendant compte de recherche originale, des perspectives (synthèses, critiques et réévaluations), des discussions (commentaires et réponses) et des communications rapides. Les documents peuvent porter sur les cellules, organismes, populations, communautés, écosystèmes ou processus qui affectent les systèmes aquatiques. Ils peuvent relever de divers domaines y compris la biologie et l'écologie des organismes marins et d'eau douce, la limnologie, l'océanographie, la physiologie, la toxicologie, la génétique, l'économie, la pathologie et la gestion. On accorde la préséance aux articles qui contribuent à la compréhension de phénomènes observés et à l'interprétation de résultats expérimentaux. On invite les auteurs à soumettre des articles ayant des objectifs précis ou relevant d'hypothèses, de concepts ou de questions vérifiables et se terminant par de solides conclusions ou d'utiles synthèses. Ces articles enrichissent, changent, mettent en question ou réorientent les connaissances acquises sur divers aspects des sciences aquatiques. On décourage toutefois la soumission d'articles simplement descriptifs (à moins qu'il ne s'agisse de nouvelles disciplines), d'application très limitée (applicables à une seule année, une seule région ou un seul taxon, portant sur un unique composé chimique, etc.); d'articles qui ne font que reprendre les principes énoncés dans des articles précédents; et d'articles rendant compte de l'application de normes sans gain de méthodologie.

Les auteurs voudront adresser leurs **manuscrits** aux directeurs scientifiques, *Journal canadien des sciences halieutiques et aquatiques*, Department of Zoology, University of Guelph, Guelph, ON N1G 2W1, Canada (519-824-4120 poste 2646; télécopieur : 519-767-9798; courriel : cjfas@uoguelph.ca).

Les contributions peuvent être en anglais ou en français. Elles doivent être originales, c.-à.-d. qu'elles ne doivent pas avoir fait l'objet d'un copyright, avoir été publiées ou soumises ailleurs, sauf sous forme abrégée ou avec le consentement du rédacteur. Le CNRC ne se tient en aucune façon responsable des opinions exprimées par un collaborateur ou des déclarations qui pourrait faire ce dernier. La publication d'annonces publicitaires, d'avis et autres n'implique pas l'endossement de leur contenu par les directeurs du Journal ou du CNRC. L'utilisation des noms de marques de commerce ne signifie aucunement une sanction du produit en question ou de la compagnie qui le fabrique.

La correspondance ayant trait aux manuscrits acceptés et les **épreuves corrigés** doivent être envoyées aux Presses scientifiques du CNRC, Conseil national de recherches du Canada, Ottawa, ON K1A 0R6, Canada.

Les Recommandations aux auteurs sont publiées une fois par année dans le premier numéro de chaque volume. Des copies sont disponibles auprès du Bureau de la rédaction aux Presses scientifiques du CNRC ou au http://jcsha.cnrc.ca. **La Politique de publication** est disponible auprès des Presses scientifiques du CNRC ou au http://www.nrc.ca/cisti/journals/.

Microfilms : On peut acheter sur microfilms de 16 ou 35 mm tous les numéros depuis 1934. Des photocopies d'articles ou de numéros individuels sont aussi en vente auprès de University Microfilms International, 330 North Zeeb Road, Ann Arbor, MI 48106, É.-U.

This journal is indexed or abstracted in / Ce journal est signalé ou résumé dans
ABSEARCH, Aquatic Sciences and Fisheries Abstracts, Biological Abstracts, Biological & Agricultural Index, CAB Abstracts, Chemical Abstracts, Current Contents, Elsevier BIOBASE, Environmental Abstracts, Environmental Periodicals Bibliography, GEOBASE, GeoRef, Life Sciences Collection, Meteorological and Geoastrophysical Abstracts, Oceanic Abstracts, PASCAL Database, and Science Citation Index.

Volume 58, Number 9, September 2001

Canadian Journal of Fisheries and Aquatic Sciences

Published by the National Research Council of Canada

Volume 58, numéro 9, septembre 2001

Journal canadien des sciences halieutiques et aquatiques

Publié par le Conseil national de recherches du Canada

Editors / directeurs scientifiques John C. Roff and Moira M. Ferguson

Assistant to the Editors / adjointe aux directeurs scientifiques **Holly Foster**

Associate Editors / directeurs scientifiques associés

M.T. Arts, *Environment Canada, Burlington*
D. Boisclair, *Université de Montréal*
U. Borgmann, *Environment Canada, Burlington*
S.E. Campana, *DFO/MPO, Dartmouth*
R. Carignan, *Université de Montréal*
D.G. Dixon, *University of Waterloo*
A.E. Dizon, *National Marine Fisheries Service, La Jolla, Calif.*
J.J. Dodson, *Université Laval*
J.A. Downing, *Iowa State University*
K.T. Frank, *DFO/MPO, Dartmouth*
D.F. Houlihan, *University of Aberdeen, Aberdeen, Scotland*
F.A. Huntingford, *University of Glasgow*
J.A. Hutchings, *Dalhousie University*
G.S. Jamieson, *DFO/MPO, Nanaimo*
B. Jonsson, *Norwegian Institute for Nature Research, Oslo, Norway*
D.M. Mason, *NOAA-Great Lakes Environmental Research Laboratory, Ann Arbor, Mich.*
A. Mazumder, *University of Victoria*
D.L. Parrish, *Vermont Cooperative Fish and Wildlife Research Unit (USGS/BRD), University of Vermont*
P. Pepin, *DFO/MPO, St. John's*
E.E. Prepas, *University of Alberta*
T.M. Powell, *University of California, Berkeley, Calif.*
T.J. Quinn II, *University of Alaska–Fairbanks, Juneau, Alaska*
T.P. Quinn, *University of Washington*
R.D. Robarts, *Environment Canada, Saskatoon*
B. Sainte-Marie, *DFO/MPO, Mont-Joli*
D.W. Schindler, *University of Alberta*
S.J. Smith, *DFO/MPO, Dartmouth*
W.G. Sprules, *University of Toronto*
R.J. Thompson, *Memorial University of Newfoundland*
C.J. Walters, *University of British Columbia*
R.D. Ward, *CSIRO Marine Research, Australia*
C.M. Wood, *McMaster University*
B. de Young, *Memorial University of Newfoundland*

NRC Research Press / Presses scientifiques du CNRC

Editor-in-chief / directeur général **Bruce P. Dancik**
Assistant Editor-in-chief / directeur général adjoint **Iain E.P. Taylor**

Journals Program / Programme des revues

(Fax / télécopieur : 613-952-7656; e-mail / courriel : research.journals@nrc.ca;
URL: http://www.nrc.ca/cisti/journals/)

Director / directrice **Aldyth Holmes**
Business Manager / gestionnaire des affaires **Paul McClymont**
Managing Editor / rédactrice administrative **Cecily M. Pearson (613-993-9099)**
Publication Officers / agentes de publication **Jennifer McColl and Lee Murphy**
Advertising / annonces publicitaires **Judy Heyman (613-993-0151)**
Reprints / tirés à part **Roxanne Landriault (613-990-2254)**
Subscriptions / abonnements **Helen Goulet (613-993-9084)**

© National Research Council of Canada 2001. World rights reserved. No part of this publication may be reproduced, stored, or transmitted in any form or by any means, without the written permission of the publisher, except as stated below.

The National Research Council of Canada grants **permission*** to individuals to make single copies of articles for personal use. Any person may reproduce short excerpts from articles in the journal for any purpose that respects the moral rights of authors, provided that the source is fully acknowledged. As a courtesy, the consent of authors of such material should be obtained directly from the author.

Authorization to reproduce items for other than personal use, as stated above, is granted by the National Research Council of Canada provided that the copyright fee of $7.00 per copy is paid directly to CANCOPY, One Yonge Street, Suite 1900, Toronto, ON M5E 1E5, Canada.

*The above permission does not extend to other kinds of copying, such as copying for general distribution, for advertising, or promotional purposes, for creating new collective works, or for resale. For such copying, arrangements must be made with the publisher, National Research Council of Canada, Ottawa, ON K1A 0R6, Canada.

© Conseil national de recherches du Canada 2001. Tous droits réservés. Il est interdit de reproduire, de sauvegarder ou de transmettre quelque partie de la présente publication dans quelque format ou de quelque façon sans le consentement écrit de l'éditeur, à l'exception des conditions suivantes.

Le Conseil national de recherches du Canada accorde aux particuliers le droit de faire des copies individuelles pour leur usage personnel. Toute personne peut reproduire de courts extraits des articles de la Revue pour autant que les droits moraux de l'auteur soient respectés et que la mention de provenance soit clairement indiquée. Il est de convenance de demander le consentement de l'auteur avant de faire mention de son travail.

On obtiendra l'autorisation de reproduire des articles à d'autres fins que l'usage personnel (tel qu'on l'a décrit ci-dessus) auprès du Conseil national de recherches du Canada pour autant que des redevances de droits d'auteurs de 7,00 $/copie soient versées au CANCOPY, 1, rue Yonge, bureau 1900, Toronto, ON M5E 1E5, Canada.

*La permission ci-dessus ne s'applique pas à la reproduction à grande échelle, telle que la reproduction aux fins de distribution générale, publicitaire ou promotionnelle, de compilation de nouveaux ouvrages collectifs ou de revente. Dans de tels cas, il faudra ratifier une entente particulière avec l'éditeur, le Conseil national de recherches du Canada, Ottawa, ON K1A 0R6, Canada.

Subscription information

The *Canadian Journal of Fisheries and Aquatic Sciences* (ISSN 0706-652X) is published monthly by the National Research Council of Canada. 2001 annual subscription rates (second class postage) for Canada are $687CAN institutional and $208CAN personal, for US & foreign $687US institutional and $208US personal. For air mail add $150US to foreign rate. Canadian customers please include 7% GST (business no. 12149 1807 RT 0275). Subscriptions are entered for the calendar year. Remittances should be made payable to the Receiver General for Canada credit NRC. Price for regular single issues: institutional, $80.00; personal, $30.00. Supplements will be priced separately. Special rates apply to members of Canadian scientific and engineering societies if they subscribe through their respective societies.

There are no refunds on cancelled subscriptions once delivery has begun. Claims for missing issues should be made within four months (N. America) or six months (outside N. America) following the date of publication. The publisher expects to supply missing issues only when losses have occurred in transit and when the reserve stock will permit. For change of address and subscription enquiries: Subscription Office, NRC Research Press, National Research Council of Canada, Ottawa, ON K1A 0R6, Canada; tel.: 613-993-9084; fax: 613-952-7656; e-mail: research.journals@nrc.ca.

Microform backfiles are available from UMI, P.O. Box 1346, Ann Arbor, MI 48106-1346, U.S.A., and Micromedia Ltd., 20 Victoria St., Toronto, ON M5C 2N8, Canada.

All issues of the *Canadian Journal of Fisheries and Aquatic Sciences* since 1996 are available on the Web. Pricing information can be found at http://www.nrc.ca/cisti/journals/.

Renseignements sur l'abonnement

Journal canadien des sciences halieutiques et aquatiques (ISSN 0706-652X) est une publication mensuelle du Conseil national de recherches du Canada. Le prix de l'abonnement annuel au Canada (courrier de deuxième classe) est de 687 $CAN pour les organismes et de 208 $CAN pour les particuliers. À l'étranger (y compris les É.-U.), le prix de l'abonnement est de 687 $US pour les organismes et de 208 $US pour les particuliers. L'expédition par avion exige des frais de 150 $US en sus du prix d'abonnement à l'étranger. Les clients du Canada doivent ajouter la TPS de 7% (numéro d'entreprise 12149 1807 RT 0275). Les abonnements sont pour l'année civile. Tout paiement doit être adressé au Receveur général du Canada, crédit du CNRC. Le prix par numéro est de 80 $ pour les organismes et de 30 $ pour les particuliers. Le prix des hors-série varie. Les membres des sociétés canadiennes de sciences et de génie bénéficient de taux préférentiels en s'abonnant via leurs sociétés respectives.

Aucun remboursement ne sera accordé ni aucune annulation acceptée une fois que les livraisons auront commencé. Les réclamations de numéros manquants devront être soumises dans les quatre mois (Amérique du Nord) ou dans les six mois (à l'extérieur de l'Amérique du Nord) suivant la date de publication. L'éditeur ne fournira de numéros manquants que si le document a été perdu pendant l'expédition et que si le stock le permet. Adressez tout changement d'adresse ou toute question quant à l'abonnement au : Bureau des abonnements, Presses scientifiques du CNRC, Conseil national de recherches Canada, Ottawa, ON K1A 0R6, Canada; téléphone : 613-993-9084; télécopieur : 613-952-7656; courriel : research. journals@nrc.ca.

On peut obtenir les fichiers rétrospectifs sur **microforme** auprès de UMI, P.O. Box 1346, Ann Arbor, MI 48106-1346, É.-U., et auprès de Micromedia Ltée, 20 rue Victoria, Toronto, ON M5C 2N8, Canada.

À partir de 1996, tous les numéros de *Journal canadien des sciences halieutiques et aquatiques* sont accessibles sur le Web. On trouvera des renseignements sur les tarifs à http://www.nrc.ca/cisti/journals.

RAPID COMMUNICATION / COMMUNICATION RAPIDE

Area-dependent patterns of finfish diversity in a large marine ecosystem

Kenneth T. Frank and Nancy L. Shackell

Abstract: The species–area relationship (SAR) is considered a cornerstone of terrestrial and freshwater ecology and conservation. It has rarely been examined in a large marine ecosystem because it has been assumed that sufficient data are lacking and (or) the scales of oceanic systems are too large. Using data drawn from fishery surveys, we show a positive relationship between the number of finfish species and the area of submarine, offshore banks on the continental shelf off eastern Canada. Banks of similar size yielded similar species richness regardless of the distance between them. Area per se had a stronger influence on species number than did habitat diversity. The slope of SAR observed is consistent with the tendency for many of the species to be highly migratory with widely dispersing offspring. This results in strong interactions among banks. The combined densities of all species increased with bank area, suggesting that larger banks have higher resources per unit area. Populations and species on larger banks should be more resilient to local extinctions relative to those on smaller banks, and natural or human-induced perturbations might be expected to impact the community structure of the small, extinction-prone populations at a faster rate.

Résumé : La relation espèces–surface (SAR) est l'une des pierres angulaires de l'écologie et de la conservation des milieux terrestres et des milieux d'eau douce. Elle a rarement été évaluée dans de grands écosystèmes marins, car on a toujours présumé qu'il n'y avait pas les données nécessaires et (ou) que l'échelle des systèmes océaniques était trop vaste. Des données d'inventaire de pêche nous ont permis d'établir une relation positive entre le nombre de poissons à nageoires et la surface des bancs sous-marins sur la plate-forme continentale au large de la côte est du Canada. Des bancs de tailles similaires possèdent des richesses spécifiques semblables, quelle que soit la distance qui les sépare. La surface par elle-même a une plus grande influence sur le nombre d'espèces que la diversité de l'habitat. La pente de SAR est en accord avec la tendance qu'ont plusieurs des espèces d'être fortement migratrices et d'avoir des petits à dispersion étendue. Cela crée d'importantes interactions entre les bancs. Les densités combinées de toutes les espèces augmentent avec la surface des bancs, ce qui mène à croire que les plus grands bancs possèdent plus de ressources par unité de surface. Les populations et les espèces des plus grands bancs devraient donc être plus résistantes aux extinctions locales que celles des plus petits bancs; les perturbations d'origine naturelle ou anthropique devraient aussi vraisemblablement affecter à un rythme plus accéléré la structure de communauté des peuplements petits et vulnérables à l'extinction.

[Traduit par la Rédaction]

Introduction

Explaining regional differences in species diversity has been a central goal of ecology, and studies of insular biota have played a prominent role in the development of theories of species diversity. It has been established that species diversity is predictably related to the area of habitat in both continental and islands faunas, i.e., the so-called species–area relationship or SAR. This holds for many animal groups (Connor and McCoy 2001). SARs have wide application. For example, they have been used to interpret how communities are organized and to explore the consequences of reserve design for the maintenance of biodiversity (Connor and McCoy 2001). SARs have been extensively studied in both terrestrial and freshwater systems. The ecological basis of the species–area relationship has generally been attributed to area per se as described in equilibrium theory of island biogeography, habitat diversity, resource concentration, and sampling phenomena (Connor and McCoy 2001). Studies that support any one of these hypotheses have been equivocal and it is probable that each contribute to patterns of species richness to varying degrees (Ricklefs and Lovette 1999).

The species–area relationship is now considered one of the cornerstones of modern ecological science and conservation biology. However, analyses of marine communities are

Received June 18, 2001. Accepted July 12, 2001. Published on the NRC Research Press Web site at http://cjfas.nrc.ca on August 2, 2001.
J16409

K.T. Frank[1] and N.L. Shackell. Department of Fisheries and Oceans, Marine Fish Division, Bedford Institute of Oceanography, P.O. Box 1006, Dartmouth, NS B2Y 4A2, Canada.

[1] Corresponding author (e-mail: frankk@mar.dfo-mpo.gc.ca).

DOI: 10.1139/cjfas-58-9-1703

generally not approached from an insular biogeographic perspective. Given the growing interest in the conservation and management of marine biodiversity, such an approach is timely. The signatory nations to the Convention of Biological Diversity (CBD) have recently agreed to make inventories, monitor changes, and make plans to conserve marine biodiversity, despite the fact that the practice of marine conservation is not as advanced as in the terrestrial environment.

However, some have argued against applying such a terrestrial-based approach to marine systems. Angel (1993) believes that the scales of oceanic systems are too large to apply terrestrial-based concepts of conservation and resource management. Gislason et al. (2000) claim that ecological theory, in general, has limited explanatory power and may not be applicable to marine ecosystems, although this opinion is not shared by all. Others have argued that because few studies of the number of species present in marine habitats exist, little else can be done in the way of application of biogeographic theory (Carlton et al. 1999). We disagree.

In the Northwest Atlantic, the offshore banks of the continental shelf have been the focus of both intensive fisheries and research. During the Wisconsin glaciation (14 000 years BP), when sea level was at a minimum, many offshore banks were oceanic islands. In the northern hemisphere, several physical processes, interacting with local bottom topography, induce clockwise currents around the edges of banks. On larger banks, these currents can manifest themselves as permanent gyres. On smaller banks, such gyres tend to be more ephemeral. These bankwide circulation features can promote the formation of characteristic faunal assemblages and are also known to influence patterns of productivity (Sinclair 1988) through limitation of dispersal to other areas. Retention of offspring at the bank level (Sinclair 1988) may help to explain the intraspecific diversity evident in some common species. Indeed, submarine offshore banks appear to exhibit a surprising level of faunal isolation. Conversely, the widespread distribution of various species suggests that dispersal and subsequent colonization have occurred since the last glaciation. The historical rates of retention and dispersal are unknown (but see Frank 1992) and have likely varied among species as the result of factors such as density dependence and oceanographic variability.

The objective of this paper was to evaluate the relationship between bank area and fish diversity on the Scotian Shelf, located off eastern Canada. We also undertake an analysis of the mechanisms by which area determines species richness.

Methods

The Scotian Shelf is composed of 10 distinct submarine banks (submerged islands) ranging in size from 500 km^2 to >10 000 km^2. In such an environment, it is both difficult to collect species numbers and to examine the processes that determine them. Fortunately, the evaluation of the SAR was made possible by the availability of detailed annual summer surveys (1970–2000) of the abundance and distribution of the finfish fauna on these banks, collected using standardized methods providing the data required (see Doubleday and Rivard 1981 for details). Because sampling effort is proportional to area in the surveys used, it is possible that fewer species are detected with lower overall sampling effort on smaller banks. We evaluated this possibility by examining species accumulation curves for each of the 10 banks. Alternatively, rarefaction curves could have been constructed for the different banks to examine the completeness of the census for each bank. We considered rarefaction inappropriate because our goal was to estimate the species number for each bank area and not the number of species per unit area. Total sampling effort (number of trawl sets) to the year 2000 for each bank was 62, 60, 63, 64, 161, 149, 172, 125, 329, and 454 for Baccaro, Roseway, LaHave, Emerald, Browns, Middle, Western, Misaine, Banquereau, and Sable, respectively.

Results and discussion

Seventy-nine species were captured across the 10 banks during the 31 years of the survey. Thirty-two families were represented. The most speciose families included Gadidae with 11 species, Cottidae (8), Cyclopteridae (7), Pleuronectidae (6), Rajidae (6), Zoarcidae (5), Stichaeaidae (4), Agonidae (3), and Clupeidae (3). These nine families contained 67% of the finfish species collected. The remaining families were represented by either one or two species.

The species-accumulation curves for each of the 10 banks exhibited an initial rapid rise in species number as samples accumulated (Fig. 1*a*). On average, the number of species collected in any given year underestimated the cumulative number of species after 31 years by 32–75%. For several of the banks, the curves tended towards an asymptote reflecting a complete census. However, for the largest banks, the census appears to have been incomplete as indicated by the lack of an asymptote. In these cases, the larger banks may have been undersampled and species richness is likely underestimated. If there is a bias in the species counts, it will manifest itself conservatively in the species–area analysis. For the analysis, we considered that the species number (S), accumulated over the 31 years of sampling for each bank, was the best available estimate of species richness.

For each bank, the number of species observed (S) was plotted against the area of the bank (A). A power relationship ($S = cA^Z$), where Z is the rate of increase of species with area and c is the expected number of species in a unit area, was then fitted to the data by least-squares linear regression following $\log_{10}$ transformation. Eighty-two percent of the variation in species number per bank was explained by area (Fig. 1). The equation describing this relationship was

$$S = 9.92A^{0.175},\ r = 0.90,\ n = 10,\ p < 0.001$$

This result supports one of the most common observations in terrestrial and freshwater ecology. Banks of similar size yielded similar estimates of species richness regardless of the distance between them. The slope of the species–area curve, Z (0.175), compared favorably with that reported for fish or invertebrates in other aquatic systems (generally less than 0.2; see references in Connor and McCoy 2001).

We examined the influence of census interval (i.e., the time interval over which the species number accumulated) on the parameters of the species–area relationship. The slope of the species–area relationship was remarkably stable after 6 years of sampling, ranging from 0.171 to 0.177 (Fig. 1*c*). This demonstrates that a single year or "instantaneous" census of marine finfish is an inferior estimate of species richness. Several others have drawn similar conclusions from

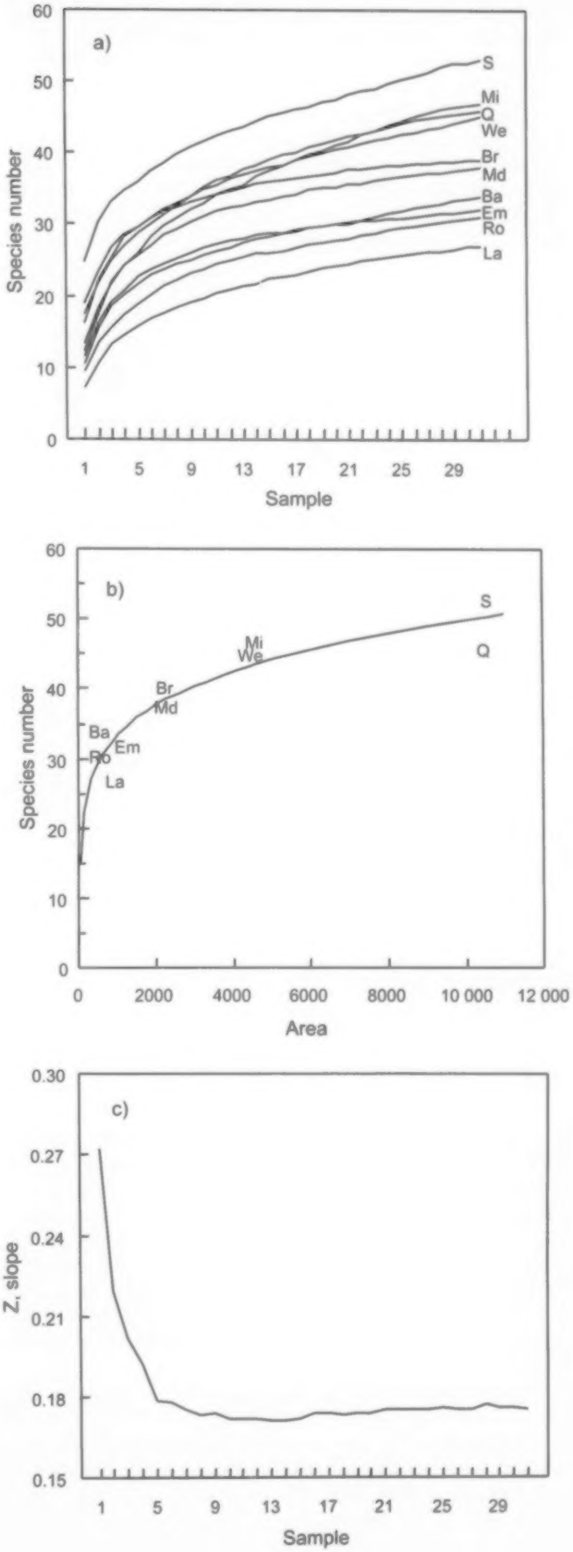


Fig. 1. (*a*) Species accumulation curves based on random addition of samples (years) to evaluate the completeness of survey sampling of finfish species richness. Samples were randomized 50 times using EstimateS software (Colwell 1997). The mean of the randomizations was then plotted against sample number. The curves represent the expected number of species without the bias of sample order. Labels refer to the different banks: Ba, Baccaro; Ro, Roseway; La, LaHave; Em, Emerald; Br, Browns; Md, Middle; We, Western; Mi, Misaine; Q, Banquereau; S, Sable. (*b*) A species–area relationship for finfish on the Scotian Shelf banks based on the cumulative species number per bank from 1970 to 2000. Data labels are centred on estimates. Bank area is in km^2. The distance between bank pairs of similar size was 51 km for Ba–Ro, 130 km for La–Em, 476 km for Br–Md, 431 km for We–Mi, and 274 km for Q–S. (*c*) Slope (Z) of the species–area relationship based on species richness estimates accumulated from 1 to 31 years across the 10 banks.

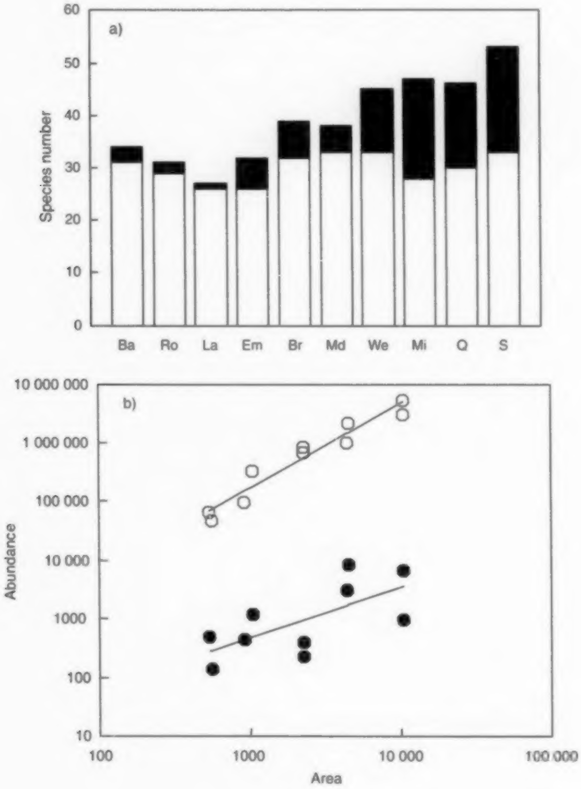
freshwater systems (see Arnott 1998 for analysis and literature review).

Low Z values are consistent with landscape scale diversity studies in which species are widely dispersed (Crawley and Harral 2001). Many of the common species studied herein are large-bodied and can easily migrate to neighboring banks. Prevailing currents can serve as immigration pathways among banks (Frank 1992) to disperse the offspring. Thus, the concepts of immigration and extinction, in the context of the equilibrium theory of island biogeography, cannot be strictly applied to strongly interactive systems such as the banks on the Scotian Shelf. Temperate marine fish communities can be considered analogous to some highly migratory bird communities occupying island complexes (see references in Ricklefs and Lovette 1999).

The functional basis for the relationship between area and species richness has traditionally been difficult to resolve. Several insights can be gained from the examination of habitat diversity indices and species richness. The most commonly used measure in terrestrial systems is maximum elevation (Ricklefs and Lovette 1999) and its counterpart in marine systems is depth variation, which has been shown to be an important determinant of marine fish community structure at large spatial scales (Mahon et al. 1998). Larger depth ranges were associated with larger banks (r = 0.84, n = 10, $p < 0.01$) requiring that partial correlation analysis be used to evaluate its effect on species number. Species number per bank was not significantly correlated with depth range when the effect of area was held constant (partial r = 0. 59, n = 10, $p > 0.05$), suggesting that depth variation was not an important determinant of species number. This result should be tempered by the fact that depth variation was relatively low across the 10 banks. For example, when we included a greater range of habitat types (i.e., areas adjacent to the banks such as slopes and basins), depth variation was a major determinant of species number when area was held constant (partial r = 0.71, n = 40, $p < 0.001$).

Alternatively, the area per se hypothesis attributes the existence of SAR to the tendency for larger areas to support larger populations that are less prone to local extinction. We found that the less common species made a greater contribution to the total species richness on the larger banks

Fig. 2. (*a*) Separation of finfish species composition into common and uncommon species based on their frequency of occurrence across the 10 banks. If a species occurred on six or more of the banks, it was considered common (represented by open bars). Species that occurred on five or fewer banks were considered uncommon (solid bars). Refer to the legend in Fig. 1*a* for full names of the banks. (*b*) Aggregate index of abundance (combined average species abundance over the 31 years of the survey) of the 34 common finfish species (open circles) and the 45 uncommon species (solid circles) in relation to bank area (km^2).



(Fig. 2*a*). If these species occur there only because the probability of local extinction is lower on larger banks, as predicted by the area per se hypothesis, we would expect population sizes, on average, to be higher on larger banks. The common species exhibited a positive correlation between abundance and area ($r = 0.94$, $n = 10$, $p < 0.001$) as did the uncommon species but to a lesser degree ($r = 0.68$, $n = 10$, $p < 0.05$; Fig. 2*b*). Indeed, it appears that on the 10 banks studied, area per se has a strong influence on species number.

A positive correlation existed between the aggregate density of all finfish species and area ($r = 0.76$, $n = 10$, $p < 0.01$) suggesting that larger banks have higher resources per unit area. In this context, resources for finfish species may include higher prey diversity and (or) productivity. Given their higher densities, larger banks may be acting as sources for populations of similar species on smaller banks.

What are the implications of SAR for marine conservation? We have shown that larger banks have more species, exhibit higher productivity per unit area, and thus are likely more resilient to perturbation. Smaller populations tend to occur on smaller banks, which would make them more prone to extinction. These results, combined with the low Z value of the species–area relationship, suggest that the complex of banks may conform to a metapopulation framework. If the conservation objective were to preserve only higher species richness, then larger banks should be protected as sources for maintaining extinction-prone populations on smaller banks (i.e., the rescue effect). If the conservation objective were to preserve population diversity and species richness, portions of both large and small banks should be protected.

The traditional single-species approach to fisheries management is slowly changing towards management at the scale of the fish community or ecosystem level. Our study represents a contribution towards this change and we encourage similar analyses of the large-scale data bases derived from research vessel surveys that exist for many coastal and continental shelf regions of the world.

Acknowledgements

We thank the staff of the Marine Fish Division at both the Bedford Institute of Oceanography and the St. Andrew's Biological Station for their sustaining commitment to the research vessel survey program of the Scotian Shelf. Drs. W.C. Leggett, D. Brickman, M. Sinclair, D. Swain, and M. Fogarty and two anonymous referees provided critical feedback on an earlier version of our work. This work was supported by the Canadian Department of Fisheries and Oceans and the Canada GLOBEC Research Partnership Program.

References

Angel, M.V. 1993. Biodiversity of the pelagic ocean. Conserv. Biol. **7**: 760–772.

Arnott, S.E. 1998. Species turnover and richness of aquatic communities in north temperate lakes. Ph.D. thesis, University of Wisconsin–Madison. UMI Microform 9904189.

Carlton, J.T., Geller, J.B., Reaka-Kudla, M.L., and Norse, E.A. 1999. Historical extinctions in the sea. Annu. Rev. Ecol. Syst. **30**: 515–538.

Colwell, R.K. 1997. EstimateS: statistical estimation of species richness and shared species from samples. Version 5 User's Guide and Application (http://viceroy.eeb.uconn.edu/estimates). University of Connecticut, Stors, Conn.

Connor, E.F., and McCoy, E.D. 2001. 2001. Species–area relationships. *In* Encyclopedia of biodiversity. Vol. 5. Academic Press, San Diego, Calif. pp. 397–411.

Crawley, M.J., and Harral, J.E. 2001. Scale dependence in plant biodiversity. Science (Washington), **291**: 864–868.

Doubleday, W.G., and Rivard, D. 1981. Bottom trawl surveys. Can. Spec. Publ. Fish. Aquat. Sci. No. 58.

Frank, K.T. 1992. Demographic consequences of age-specific dispersal in marine fish populations. Can. J. Fish. Aquat. Sci. **49**: 2222–2231.

Gislason, H.M., Sinclair, M., Sainsbury, K., and O'Boyle, R.N. 2000. Symposium overview: incorporating ecosystem objectives within fisheries management. ICES J. Mar. Sci. **57**: 468–475.

Mahon, R., Brown, S.K., Zwanenburg, K., Atkinson, D.B., Buja, K.R., Claflin, L., Howell, G.D., Monaco, M.E., O'Boyle, R.N., and Sinclair, M. 1998. Assemblages and biogeography of demersal fishes of the east coast of North America. Can. J. Fish. Aquat. Sci. **55**: 1704–1738.

Ricklefs, R.E., and Lovette, I.J. 1999. The roles of island area per se and habitat diversity in the species–area relationships of four Lesser Antillean faunal groups. J. Anim. Ecol. **68**: 1142–1160.

Sinclair, M. 1988. Marine populations: an essay on population regulation and speciation. University of Wisconsin Press, Seattle, Wash.

Influence of sediment biofilm on the behaviour of aluminum and its bioavailability to the snail *Lymnaea stagnalis* in neutral freshwater

S. Ballance, P.J. Phillips, C.R. McCrohan, J.J Powell, R. Jugdaohsingh, and K.N. White

Abstract: An important influence on the behaviour, bioavailability, and toxicity of Al in neutral freshwater is its ability to form complexes with organic material such as humic acids and extracellular polymeric substances (EPS). This paper examines the influence of EPS, secreted by a natural bacterial biofilm associated with a pebble substrate ("sediment biofilm") and by the snail *Lymnaea stagnalis*, on the behaviour of Al in the water column and its bioavailability to the snail. Both sediment biofilm and snails were a significant source of aqueous EPS. Added Al stimulated the production of EPS by the snail but not by bacterial biofilm. Repeated elevation of the concentration of Al in the water by 500 μg Al·L^{-1} but not 100 μg Al·L^{-1} over 10 days resulted in a progressive rise of Al in the water column in the absence but not in the presence of sediment biofilm. Up to 150 μg Al·cm^{-2} was associated with the sediment biofilm, and we suggest that sediment is a significant "sink" for aqueous Al. EPS avidly binds colloidal Al, and we propose that the sediment biofilm is an important influence on the behaviour and bioavailability of Al in running waters when amounts of humic substances are low.

Résumé : La capacité de l'aluminium de former des complexes avec du matériel organique, tel que les acides humiques et les substances polymériques extracellulaires (EPS) influence de façon significative son comportement, sa biodisponibilité et sa toxicité en eau douce neutre. Nous examinons ici l'influences des EPS sécrétées par un biofilm bactérien naturel sur un substrat de cailloux (« biofilm des sédiments ») et par le gastéropode *Lymnaea stagnalis* sur le comportement de l'aluminium dans la colonne d'eau et sa biodisponibilité au gastéropode. Tant le biofilm des sédiments que les gastéropodes sont des sources significatives d'EPS aqueuses. L'addition d'aluminium stimule la production d'EPS par le gastéropode, mais pas par le biofilm bactérien. Des accroissements répétés des concentrations d'aluminium dans l'eau de l'ordre de 500 μg Al·L^{-1}, mais non de 100 μg Al·L^{-1}, sur une période de 10 jours ont causé une augmentation de l'aluminium dans la colonne d'eau en l'absence du biofilm des sédiments, mais non en sa présence. Des quantités d'aluminium pouvant atteindre 150 μg Al·cm^{-2} sont associées au biofilm des sédiments et les sédiments sont, croyons-nous, un puits important pour l'aluminium en forme aqueuse. Les EPS se lient facilement à l'aluminium colloïdal; nous suggérons donc que le biofilm des sédiments a une influence considérable sur le comportement et la biodisponibilité de l'aluminium en eau courante, lorsque les concentrations de substances humiques sont basses.

[Traduit par la Rédaction]

Introduction

Although highly toxic, Al was thought to be unavailable to biological systems in freshwaters at neutral pH (Driscoll and Schecher 1989) owing to the formation of insoluble particulates and complexation with organic material. We have recently shown that mucus biofilm, secreted by the pond snail *Lymnaea stagnalis*, binds Al and enhances its rate of removal from the water column (Jugdaohsingh et al. 1998). However, many benthic invertebrates graze on the secreted biofilm. We hypothesise that grazing accounts for the marked accumulation (Elangovan et al. 1997) and sublethal toxicity (Truscott et al. 1995; Campbell et al. 2000) of Al to *L. stagnalis* at environmental concentrations in neutral waters.

A biofilm may be defined as a gelatinous extracellular polymeric substance (EPS) of biological origin containing a consortium of bacteria, fungi, and algae. Biofilms are present at the solid–aqueous interface throughout the aquatic environment (Lappin-Scott et al. 1993) and typically at the sediment–water interface (Davidson et al. 1997). Biofilms are formed from mucus secreted by benthic invertebrates including gastropods (Davies and Hawkins 1998) and from the synthesis of capsular and extracellular polymers by bacteria and algae (Costerton et al. 1987; Leppard 1995). Biofilms are thought to have an important influence on the cycling of

Received July 5, 2000. Accepted June 1, 2001. Published on the NRC Research Press Web site at http://cjfas.nrc.ca on August 10, 2001.
J15847

S. Ballance, P.J. Phillips, C.R. McCrohan, and K.N. White.[1] School of Biological Sciences, University of Manchester, 3.614 Stopford Building, Oxford Road, Manchester M13 9PT, U.K.
J.J Powell and R. Jugdaohsingh. Gastrointestinal Laboratory, The Rayne Institute, St. Thomas' Hospital, London SE1 7EH, U.K.

[1] Corresponding author (e-mail: keith.white@man.ac.uk).

DOI: 10.1139/cjfas-58-9-1708

trace metals such as Zn and Fe (Davidson et al. 1997) and to facilitate the entry of these metals into the aquatic food chain (Bremer and Loutit 1986*a*, 1986*b*).

In the natural aquatic environment, the benthic substrate provides an enormous surface area for colonisation by biofilm-producing microorganisms. For example, we have calculated that a pebble substrate of median diameter 3.1 mm has a surface area of approximately 250 mm^2·g^{-1}. The potential influence of sediment-associated biofilm ("sediment biofilm") on the behaviour of Al in the water column is therefore likely to be marked, although it has not been examined or quantified. In this paper, we examine the hypothesis that sediment biofilm exerts a significant influence on the behaviour of freshly neutralised Al in freshwaters and its bioavailability to grazing organisms such as *L. stagnalis*.

First, we confirmed that sediment would attract a biofilm and hence EPS deposition, both in the presence and in the absence of snails. Waterborne EPS was used as a measure of ongoing deposition, while total deposition was assessed at the end of the experiment by analysis of the amount of EPS bound to the sediment. Second, we investigated the concentration of Al in the water column at short intervals during the first 24 h and daily intervals thereafter for a total of 10 days. The aim was to investigate the relationship between EPS deposition from a microbial biofilm and (or) snails and the concentration of Al retained in the water column. Finally, at the end of the experiment, we measured the Al bound to the sediment and accumulated by the snails. To address the hypothesis that sediment biofilm has a significant influence on the behaviour and bioavailability of Al, we then examined the relationship between the above variables (Al, microbial EPS, snail-derived EPS, and snails).

Materials and methods

Only high-density plastic materials were used and were all acid washed for 24 h in 10% nitric acid (BDH "Analar," Poole, U.K.) prior to their use.

Experimental design

A total of 24 tanks were set up (2 × 12). Tanks contained added Al at either 0, 100, or 500 µg·L^{-1} (i.e., three treatments) and either pebbles alone, pebbles plus snails, snails alone, or neither pebbles nor snails (i.e., four tank conditions). Each treatment/condition combination was duplicated. The pebbles (composed of quartz and obtained from Gaye Life Aquatics, Manchester, U.K.) were 1–8 mm in diameter (median 3.1 mm) and completely covered the bottom of the tank (5.7×10^{-2} m^2) at an even depth of 2 cm to give a total volume of 1.5×10^{-3} m^3 pebbles per tank. The pebbles were first washed with 10% nitric acid (BDH "Analar," Poole, U.K.) to remove any adsorbed Al or organic material. The pebble sediment was then covered with standard snail water (SSW) (Thomas et al. 1975) for 5 days, as microscopical examination revealed that this was sufficient to allow the development of a bacterial biofilm. After 5 days, the SSW was carefully removed and 3 L of fresh SSW was added to each tank and left a further 24 h. Snails were added at this time; five snails (1–3 cm shell length, Blades Biological, Kent, U.K.) were added to each tank, where appropriate. Aluminum was added from a stock Al^{3+} solution (500 mg·L^{-1}, added as the nitrate and acidified to pH 2.0 to maintain the metal in solution) to elevate the concentration by 0, 100, or 500 µg·L^{-1} in five repeat applications at 48-h intervals over a period of 10 days from the start of the experiment (i.e., just after the snails were added). A 10-day exposure period was selected, as previous studies (Elangovan et al. 1997; Jugdaohsingh et al. 1999) have revealed marked changes in EPS and Al concentration in the water and Al in snail soft tissue over this period. The water was not changed during the course of the experiment but was continuously aerated and maintained at 10°C. The pH was monitored daily and adjusted to 7 ± 0.5 by the addition of 1 M ultrapure nitric acid (Romil "Sp A," Cambridge, U.K.) or 1 M sodium hydroxide (BDH "Aristar," Poole, U.K.) when necessary. The tanks were subjected to a 12 h light : 12 h dark regime and, after the start of the experiment, snails were not fed. Prior to the experiment, snails were acclimatised for 10 days under identical conditions in one large tank containing 10 L of SSW and were fed washed lettuce ad libitum.

For the measurement of total Al concentration in the water, one 10-mL water sample was removed from each tank at the start, at 0.5, 1, 2, 4, and 24 h, and every 24 h thereafter until the end of the experiment. One sample was also removed immediately following the addition of each fresh dose of Al. All of these samples were immediately acidified with 100 µL of concentrated ultrapure nitric acid (Romil "Sp A," Cambridge, U.K.). For measurement of total polysaccharide concentration in the water, one 10-mL water sample was removed from each tank every 48 h from the start of experiment and frozen at –80°C. At the end of the experiment (10 days), five snails were sampled from each tank containing snails and immediately snap frozen in liquid nitrogen prior to analysis of Al in their soft tissues. Three replicate samples of sediment biofilm were taken from each tank (six in total for each treatment/condition) for analysis of Al and a further six for analysis of polysaccharide. Each of these sediment biofilm samples (20 g) was contained in a separate perforated plastic pot (diameter 25 mm, height 20 mm) buried just beneath the sediment surface. Sediment biofilms sampled for polysaccharides were immediately frozen and stored at –80°C until analysis.

Analysis

The concentration of Al in the water samples, digested soft snail tissue, and sediment biofilm samples was measured by inductively coupled optical emission spectroscopy (ICPOES) as described previously (Burden et al. 1995). The detection limit was 2 µg Al·L^{-1}. Soft snail tissue and sediment biofilm were initially gently washed in distilled water. The soft snail tissue was then dried. Both the sediment biofilm and the soft snail tissue (approximately 200 mg) were then digested in 5 mL of concentrated nitric acid (Romil "Sp A," Cambridge, U.K.) and 5 mL of concentrated hydrogen peroxide (BDH "Aristar," Poole, U.K.) at room temperature. The resulting digest was then diluted at a ratio of 1:3 with ultrapure water.

The concentration of polysaccharide in the remaining water samples was measured by the periodic acid Schiff (PAS) method as described previously (Jugdaohsingh et al. 1998). However, this time, the PAS reaction was calibrated using purified snail glycoprotein isolated from the foot mucus of *L. stagnalis* in our laboratory. The detection limit was 0.5 mg·L^{-1}.

Biofilm was extracted from the sediment biofilm samples by repeated shaking with two 5-mL aliquots of 6 M guanidinium hydrochloride (GuHCl) (purified using activated charcoal) containing 0.1 M Tris and 5 mM EDTA (pH 8) for 24 h at room temperature. The extraction was then repeated with the addition of 10 mM dithiothreitol to the GuHCl at 37°C for 2.75 h (Carlstadt et al. 1985). No PAS-positive material remained on the pebbles after the above treatments, according to the PAS assay of Pearse (1960). The extracts were centrifuged at 2050 × *g* for 20 min to remove any snail faeces and the supernatant dialysed against three changes of ultrapure water at 4°C over 36 h. The dialysate was immediately frozen at –80°C and freeze-dried. The concentration of PAS-positive material in the freeze-dried samples was measured as above.

Fig. 1. Mean concentration of Al in the water column over 10 days ($n = 2$ from duplicate tanks) following no addition of Al (◆) and repeated (five times) 48-h additions of Al to give an initial concentration of 100 (■) or 500 $\mu g \cdot L^{-1}$ (○) in the (*a*) presence and (*b*) absence of sediment biofilm in the absence of *L. stagnalis* and in the (*c*) presence and (*d*) absence of sediment biofilm in the presence of *L. stagnalis*.

Data analysis

The concentration of Al in the water at each sampling interval was taken as the mean value calculated from combined measures for duplicate tanks. These means were then transformed by their natural logarithm to a straight line to fit the observed, approximately exponential, decay. For each treatment and tank condition, linear regression was applied to each 48-h time period after the addition of Al to calculate the gradient of log-transformed loss of Al from the water column. For the four tank conditions, gradients for each treatment were compared using analysis of covariance (ANCOVA). This gave an assessment of the change in rate of loss of Al from the water column (differences in slope) and the change in the relative amount of Al in the water column over time (differences in elevation).

The effects of each tank condition, treatment (added Al), and duration of Al exposure on the concentration of polysaccharide in tanks were analysed using three-way factorial analysis of variance (ANOVA). ANCOVA was not used because the change in the measured concentration of polysaccharide in the water column did not fit a straight line.

The effects of snails on the concentration of polysaccharide and Al in the sediment biofilm and the effects of sediment biofilm on the concentration of Al in the snail tissues were analysed using 2 × 3 factorial ANOVA. Where significant differences were observed between duplicate tanks, the within-tank mean square was used as an estimate of error. Where differences were not significant, the between- and within-tank components were combined. The probability of differences was taken as significant ($P < 0.05$), highly significant ($P < 0.01$), and very highly significant ($P < 0.001$).

The average concentration of Al in the water column during the 10-day experiment for each tank condition and treatment combination was calculated so that, in turn, concentration factors and proportionality of Al between the sediment biofilm and the tissue of the snail could be calculated. Briefly, the mean concentration of Al in water for each of the 48 h periods after the addition of Al was first calculated by summing together the actual measured concentrations at 0, 24, and 48 h after the addition of Al. The mean of these five mean values for each tank and treatment combination was then taken as the overall mean concentration in the water column. Concentration factors are therefore expressed as the ratio of the amount of Al in the tissue (micrograms per kilogram) or sediment biofilm (micrograms per square centimetre) to the average in the water column (micrograms per litre).

Results

Following each 48-h addition of the stock solution to the water column to elevate the concentration of Al to 500 $\mu g \cdot L^{-1}$, the initial concentration decreased at an approximately exponential rate at least for the first 96 h in all of the four tank conditions (Fig. 1). In tanks that contained no sediment, with or without snails (Figs. 1*b* and 1*d*), and where Al was repeatedly dosed to give an initial concentration of 500 $\mu g \cdot L^{-1}$ every 48 h for 10 days, the rate of Al loss from the water column decreased ($P < 0.01$) over the course of the experiment with a concurrent increase ($P < 0.01$) in the relative amount of Al in the water column compared with tanks where the same amount of Al was added but that contained a sediment biofilm (Figs. 1*a* and 1*c*). In tanks where Al was added to give an initial concentration of 100 $\mu g \cdot L^{-1}$, the metal was also lost from the water column but at a much lower rate. There was no significant difference in the rate of Al loss from the water column over 10 days for any of the tank conditions (Fig. 1). There was a small but significant ($F_{[3,15]} = 5.23$, $P < 0.05$) increase in the relative amount of Al in the water column in tanks containing no sediment biofilm and repeatedly dosed to give an initial concentration of 500 $\mu g \cdot L^{-1}$. This suggests that saturation of the relatively small surface area available for adsorption of Al occurs in the absence of sediment biofilm. In tanks where no Al was added, there was no significant change in the amount or rate of Al loss from the water column over the course of the experiment (Fig. 1).

Following washing of the pebbles and the initial addition of the SSW, waterborne EPS concentrations were undetectable (results not shown). At the start of the experiment, after a total of 6 days of incubation in SSW, EPS was detectable (>0.5 $mg \cdot L^{-1}$) in all treatments and increased to a highly significantly level ($P < 0.01$) in all tanks with snails and (or) sediment biofilm over the 10-day course of the experiment (Fig. 2). In tanks where Al was added to give an initial concentration of 100 or 500 $\mu g \cdot L^{-1}$, significantly ($P < 0.05$) more EPS was present in the water column in the presence than in the absence of snails (Figs. 2*b* and 2*c*). The presence of a sediment biofilm and snails also led to a highly significant ($P < 0.01$) increase in the concentration of EPS in the water compared with snails alone, probably as a result of bacteria in the water derived from sediment (Figs. 2*b* and 2*c*).

No significant difference was found in the concentration of EPS in the sediment biofilm between duplicate tanks ($F_{[6,24]} = 0.67$, $P > 0.05$), so between- and within-tank components were combined. The quantity of the EPS in the sediment biofilm was, however, very highly significantly greater ($F_{[1,30]} = 34.6$, $P < 0.001$) in the presence of snails compared with just sediment biofilm alone (Fig. 3). The addition of Al to water did not lead to a significant increase in the amount of EPS in the sediment either in the presence or in the absence of snails.

In contrast with the measured EPS in the sediment biofilm, differences between duplicate tanks in the amount of Al in the sediment were significant ($F_{[6,24]} = 3.49$, $P < 0.05$). Consequently, the within-tank mean squares were used as estimates of error in factorial ANOVA. The presence of snails had a very highly significant ($P < 0.001$) effect, causing a decrease in the amount of Al in the sediment biofilm compared with tanks with no snails (Fig. 4). There was, however, no significant difference in the amount of Al in the sediment when the metal was repeatedly added to give an initial concentration of either 100 or 500 $\mu g \cdot L^{-1}$ either in the presence or in the absence of snails ($F_{[2,24]} = 3.12$, $P > 0.05$). However, some saturation of the sediment biofilm is indicated, as the concentration factor of Al in the sediment biofilm, with respect to the calculated average concentration of Al in the water over the course of the experiment, decreased from 4.5 ×

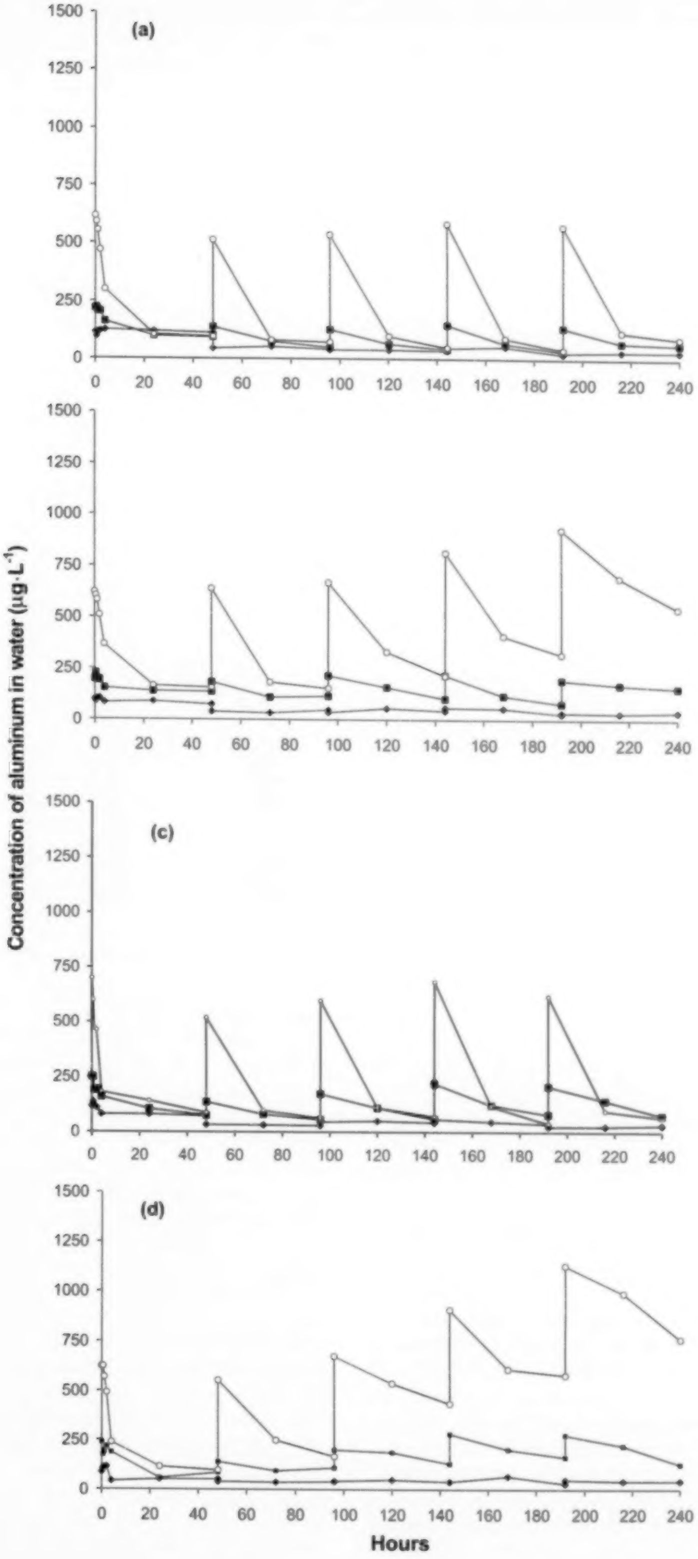
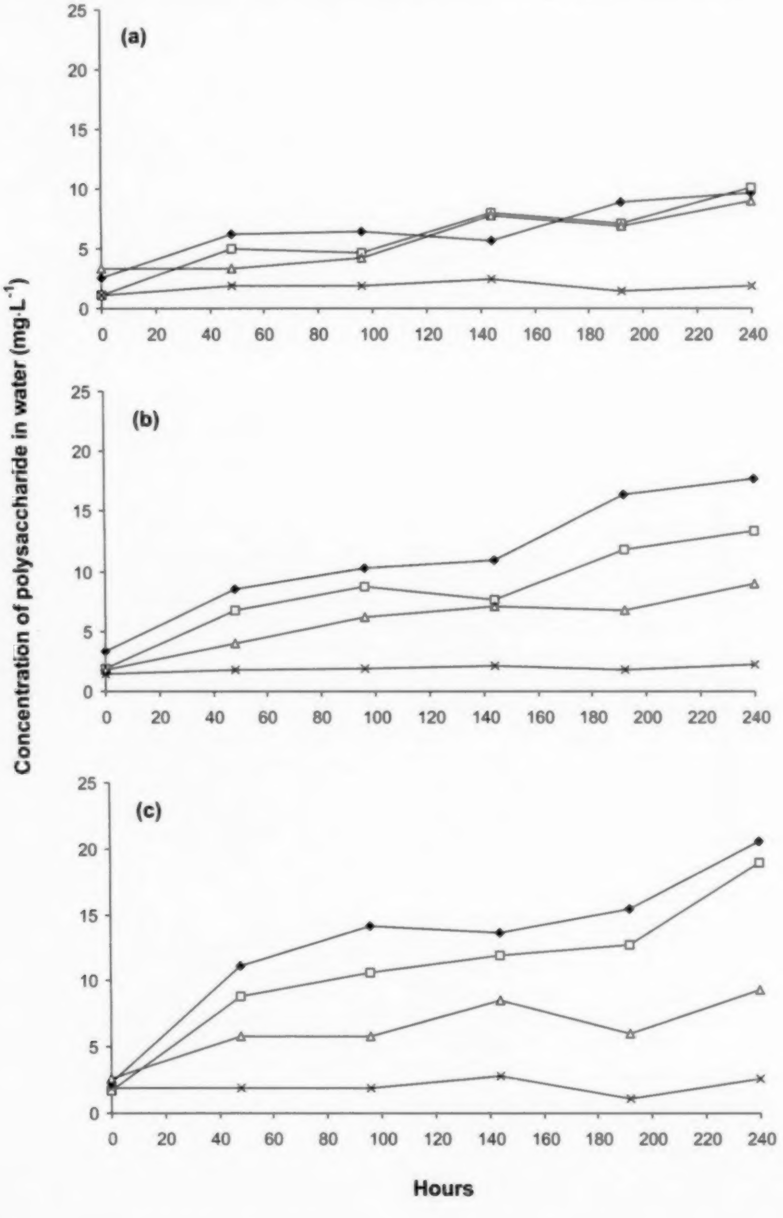

(a)
(c)
(d)
Concentration of aluminum in water (µg·L⁻¹)
Hours
0
250
500
750
1000
1250
1500
0
20
40
60
80
100
120
140
160
180
200
220
240

Fig. 2. Mean concentration ($n = 2$ from duplicate tanks) of polysaccharide in the water column in the presence of *L. stagnalis* and sediment biofilm (◆), *L. stagnalis* only (□), sediment biofilm only (△), and water only (×) (*a*) with no added Al and following repeated (five times) additions of Al to give an initial concentration of (*b*) 100 μg·L^{-1} or (*c*) 500 μg·L^{-1} over 10 days.



10^3 in the absence of added Al to 1.6×10^3 following repeated additions of Al to give an initial concentration of 500 μg·L^{-1}.

Differences between duplicate tanks in the concentration of Al in the snail tissues were highly significant ($F_{[6,36]} = 4.26$, $P < 0.01$). Again, the within-tank mean squares were used as an estimate of error in factorial ANOVA. In the presence of a sediment biofilm, *L. stagnalis* showed a markedly lower ($P < 0.001$) body concentration of Al than in the absence of a sediment biofilm. Proportionality between tissue Al and exposure concentration was observed at the end of the 10-day experiment ($R^2 = 0.8$) (Fig. 5). Concentration factors of Al in the snails compared with average exposure concentration in the SSW ranged from 1×10^2 to 8×10^2.

Discussion

Our results show that the sediment is an important control

Fig. 3. Mean concentration (±1 SD, $n = 6$) of polysaccharide in the sediment biofilm (per square centimetre of sediment surface area) with no added Al or following repeated (five times) additions of Al to give an initial concentration of 100 or 500 $\mu g \cdot L^{-1}$ in the presence (open bars) and absence (solid bars) of *L. stagnalis* over 10 days.

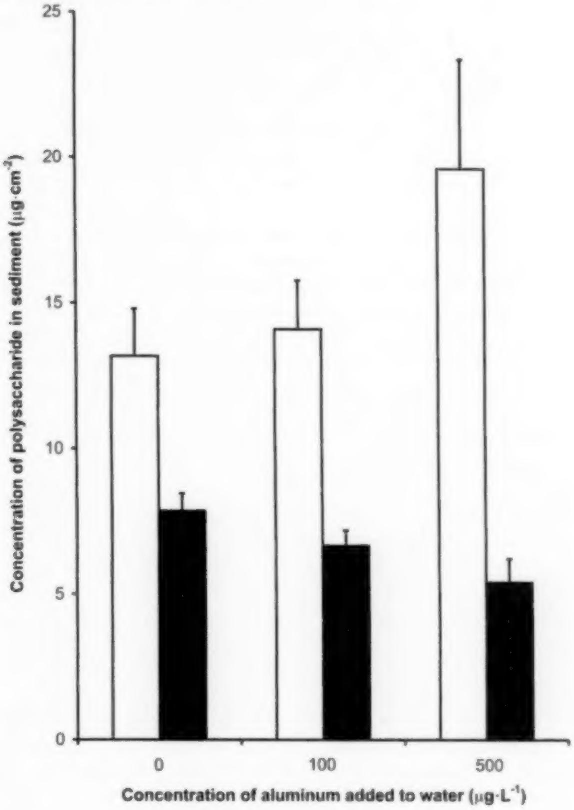
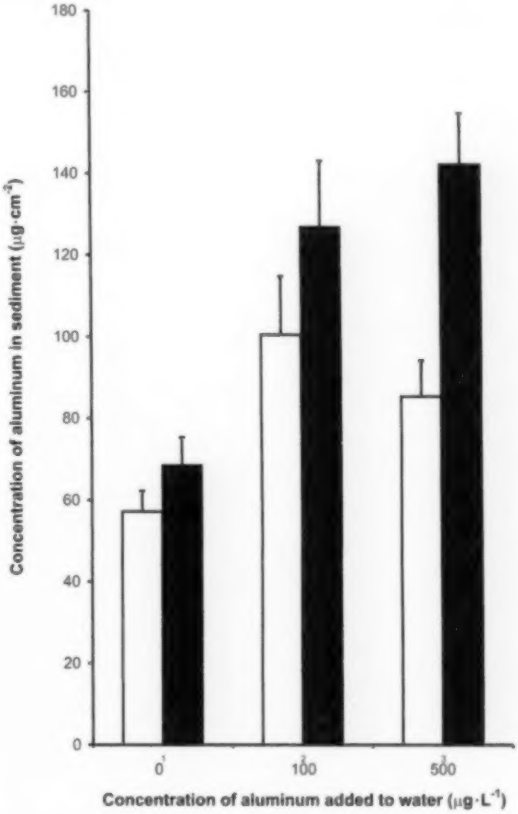


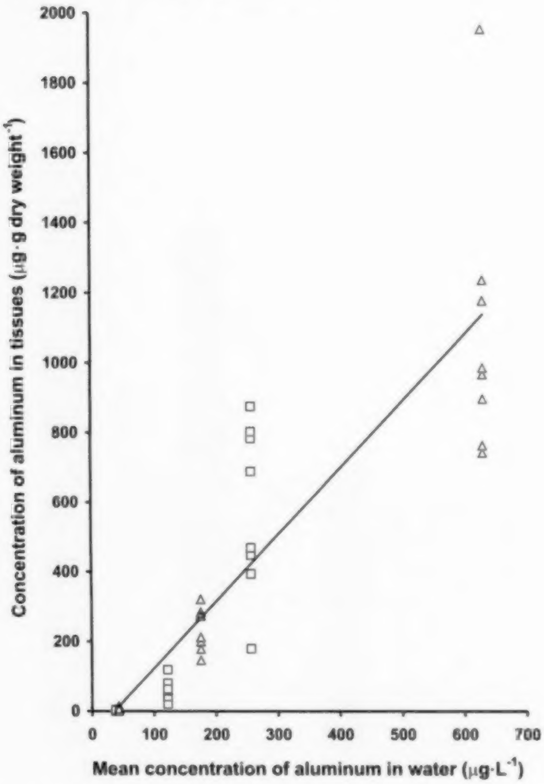
Fig. 4. Mean concentration (±1 SD, $n = 6$) of Al associated with the sediment biofilm (per square centimetre of sediment surface area) with no added Al, and following repeated (five times) additions Al to give an initial concentration of 100 or 500 $\mu g \cdot L^{-1}$ in the presence (open bars) and absence (solid bars) of *L. stagnalis* over 10 days.



on the amount of Al in the water column, and we suggest that this is due to the biofilm that is present on the surface of the substrate. We have previously shown (Jugdaohsingh et al. 1998) that mucus biofilm produced by *L. stagnalis* binds Al and so reduces the Al concentration in the water column, while other studies have revealed that bacterial biofilms sequester heavy metals such as Cu and Zn (Davidson et al. 1997). The pH–redox equilibrium equation speciation model (Parkhurst et al. 1980) predicts that Al in excess of 80 $\mu g \cdot L^{-1}$ exists as either colloidal or insoluble $Al(OH)_3$ in neutral SSW. It is therefore likely that the sediment biofilm preferentially localises the colloidal Al component that predominates soon after neutralisation of the acidic (pH 2.0) stock solution following repeated additions of Al to give an initial concentration of 100 or 500 $\mu g \cdot L^{-1}$. It is proposed that colloids of Al bind avidly to EPS produced by a biofilm due to the electrostatic association of positively charged colloids with polyanionic groups (predominantly the carboxyl and hydroxyl functional groups) on the acidic carbohydrate component (Lion et al. 1988). A similar mechanism is well known for humic acids (Jekel 1986; Gjessing et al. 1989). Light-scattering experiments (S. Ballance, unpublished data) indicate that some of the Al in our experiments will exist in the form of colloidal hydroxypolymers after 24 h, with only a weak positive charge (Smith and Hem 1972). Binding of these larger colloids to the biofilm may therefore result from entrapment within the gelatinous EPS matrix. Entrapment has been observed for Fe in a heavily polluted acid mine drainage system where metal load is often above saturation (Boult 1996). However, in unpolluted waters, abundant soluble and insoluble organic matter, including biofilm, probably binds Al before it has a chance to polymerise, and therefore, entrapment may not be important.

Humic acids are the dominant organic component in lotic waters and are crucial in controlling the behaviour of metals (Livens 1991). However, large amounts of carbohydrate are also present in freshwaters (comprising >10% of dissolved organic carbon; Gremm and Kaplan 1998), including attached to the sediment in the form of an EPS biofilm. We suggest that in running waters where the wetted perimeter to discharge ratio is high and the humic acid load low, binding of Al to EPS associated with the substrate is an important influence on the metal's transport and behaviour. The ability

Fig. 5. Concentration of Al in the whole soft tissues of *L. stagnalis* with no added Al and following repeated (five times) additions of Al to give an initial concentration of 100 or 500 μg·L^{-1} in the absence (△) and presence (□) of sediment biofilm over 10 days. The Al concentrations in the water are the average over the 10-day exposure period.



of sediment biofilm to remove Al from the water column is likely, under certain conditions, to markedly influence the behaviour and fate (including subsequent diagenesis and mineral morphology) of Al in natural freshwaters.

EPS remaining in the water column is thought to localise colloidal Al, thus holding it in complexed suspension or solution (Jugdaohsingh et al. 1998). Release of soluble and insoluble EPS from the sediment biofilm into the water may therefore reduce the total amount of Al lost from the water column as a result of binding to either humic acids or biofilm.

EPS secreted as a gelatinous sheath by many phytoplankton bind heavy metals (for a review, see Green and Darnal 1990) and are thus likely to localise Al also. The level of primary production in the water column is therefore likely to be a greater influence on the behaviour of Al in eutrophic standing waters than sediment biofilm. In contrast, humic substances are likely to be a more important control on Al behaviour in oligotrophic standing waters, as primary productivity will be low.

Snails significantly increased the amount of EPS both in the water column and associated with the sediment. This is likely to be from mucus secreted from the foot during locomotion. Furthermore, nutrients released by the snail as excretory products may stimulate the growth of bacterial biofilm (e.g., Uhlinger and White 1983); the relationship between EPS and the presence of snails may therefore be indirect. Further work is required to clarify the role of snails in the production of bacterial EPS, as the PAS stain was calibrated against purified snail glycoprotein, which may have underestimated the amount of bacterial EPS in the water column.

Aluminum appears to stimulate mucus production by the snail, as the amount of EPS in the water column increased following exposure to added metal. Increased secretion of mucus has been shown to occur in other invertebrates in response to heavy metal induced stress (Hietanen et al. 1988; Wicklum and Davies 1996) and to Al in leeches (S. Ballance, unpublished data) and fish (Mueller et al. 1991; Wilson et al. 1994).

The binding of heavy metals to bacterial EPS has been proposed as an important route of entry into the food chain (Mittleman and Geesey 1985; Wolfaardt et al. 1994). Mucus production in gastropods is estimated to account for 30–80% of their energy intake (Davies and Hawkins 1998), and therefore, snails actively scavenge their own and other biofilm to regain some of this lost energy plus associated microorganisms and trace elements. However, in this study, snails exposed to added Al at 100 and 500 μg·L^{-1} accumulated less Al when in the presence of sediment biofilm. It is possible that the larger surface area of substrate available to bind Al resulted in a lower concentration per unit area of biofilm. The inverse relationship between the amount of EPS and Al associated per unit area of sediment supports this view. Furthermore, the sediment provides a more uneven surface on which to graze compared with the smooth surface of the tanks containing no sediment, and some parts of the sediment would have been inaccessible to the snails, thus reducing feeding efficiency.

Humic substances also contain carbohydrate functionality (Fox 1983), and studies are under way to compare the effect of EPS and humics on the behaviour and bioavailability of Al in running and standing waters. Finally, the binding of Al to the sediment biofilm is only likely to be important in the biogeochemical cycling of Al if the metal is not rapidly released. Studies are therefore planned to examine the half-life of Al associated with biofilms transferred to the natural environment.

Acknowledgements

We thank the Special Trustees of St. Thomas' Hospital for their continued support, the Wellcome Trust for provision of the ICPOES, and Dr. R S Callow for advice and assistance with the statistical analysis. S.B. is in receipt of a BBSRC research studentship and P.J.P. was partially funded by the European Social Fund.

References

Boult, S. 1996. Fluvial metal transport near sources of acid minedrainage: relationships of soluble, suspended and deposited metal. Miner. Mag. **60**: 325–335.

Bremer, P.J., and Loutit, M.W. 1986*a*. Bacterial polysaccharide as a vehicle for the entry of Cr(III) to a food chain. Mar. Environ. Res. **20**: 235–248.

Bremer, P.J., and Loutit, M.W. 1986*b*. The effect of Cr(III) on the form and degradability of a polysaccharide produced by a bacterium isolated from a marine sediment. Mar. Environ. Res. **20**: 249–259.

Burden, T.J., Powell, J.J., Taylor, P.D., and Thompson, R.P.H. 1995. Optimal accuracy, precision and sensitivity of inductively coupled plasma optical emission spectrometry: bioanalysis of aluminium. J. Anal. At. Spectrom. **10**: 259–266.

Campbell, M.M., White, K.N., Jugdaohsingh, R., Powell, J.J., and McCrohan, C.R. 2000. Effect of aluminum and silicic acid on the behaviour of the freshwater snail *Lymnaea stagnalis*. Can. J. Fish. Aquat. Sci. **57**: 1151–1159.

Carlstadt, I., Sheehan, J.K., Corfield, A.P., and Gallagher, J.T. 1985. Mucous glycoproteins: a gel of a problem. Essays Biochem. **20**: 40–76.

Costerton, J.W., Cheny, K.J., Geesey, G.G., Ladd, T.I., Nickel, J.C., Dasgupta, M., and Marie, T.J. 1987. Bacterial biofilm in nature and disease. Annu. Rev. Microbiol. **41**: 435–464.

Davidson, W., Fones, G.R., and Grime, G.W. 1997. Dissolved metals in surface sediment and a microbial mat. Nature (Lond.), **387**: 885–888.

Davies, M.S., and Hawkins, S.J. 1998. Mucus from marine molluscs. Adv. Mar. Biol. **34**: 1–71.

Driscoll, C.T., and Schecher, W.D. 1989. Aqueous chemistry of aluminium. *In* Aluminium and health — a critical review. *Edited by* H.J. Gileman. Marcel Dekker, New York. pp. 27–65.

Elangovan, R., White, K.N., and McCrohan, C.R. 1997. Bioaccumulation of aluminium in the freshwater snail *Lymnaea stagnalis* at neutral pH. Environ. Pollut. **96**: 29–33.

Fox, L.E. 1983. Geochemistry of humic acid during estuarine mixing. *In* Aquatic and terrestrial humic materials. *Edited by* R.F. Christman and E. Gjessing. Ann Arbor Science, Ann Arbor, Mich.

Gjessing, E., Riise, G., Petersen, R.C., and Andruchow, E. 1989. Bioavailability of aluminium in the presence of humic substances at low and moderate pH. Sci. Total Environ. **8**: 683–690.

Green, B., and Darnal, D.W. 1990. Microbial oxygenic photoautotrophs (cyanobacteria and algae) for metal-ion binding. *In* Microbial mineral recovery. *Edited by* M. Ehrlich and C.L. Brierley. McGraw-Hill, New York. pp. 277–302.

Gremm, T.H., and Kaplan, L.A. 1998. Dissolved carbohydrate concentration, composition, and bioavailability to microbial heterotrophs in stream water. Acta Hydrochim. Hydrobiol. **26**: 197–171.

Hietanen, B., Sunila, E., and Kristoffersson, R. 1988. Toxic effects of zinc on the common mussel *Mytlus edulis* L. (Bivalvia) in brackish water. I. Physiological and histopathological studies. Ann. Zool. Fenn. **25**: 341–347.

Jekel, M.R. 1986. Interactions of humic acids and aluminium salts in the flocculation process. Water Res. **20**: 535–542.

Jugdaohsingh, R., Campbell, M.M., Thompson, R.P., McCrohan, C.R., White, K.N., and Powell, J.J. 1998. Mucus secretion by the freshwater snail *Lymnaea stagnalis* limits aluminium concentrations of the aqueous environment. Environ. Sci. Technol. **32**: 2591–2595.

Lappin-Scott, H.M., Jass, J., and Costerton, J.W. 1993. Microbial biofilm formation and characterisation. *In* Microbial films: formation and control. *Edited by* H.M. Lappin-Scott and J.W. Costerton. SAB Press, London, U.K. pp. 1–73.

Leppard, G.G. 1995. The characterization of algal and microbial mucilages and their aggregates in aquatic ecosystems. Sci. Total Environ. **165**: 103–131.

Lion, L.W., Schuler, M.L., Hsieh, K.M., and Ghiorse, W.C. 1988. Trace metal interactions with microbial biofilms in natural and engineered systems. CRC Crit. Rev. Environ. Contam. **17**: 273–306.

Livens, F.R. 1991. Chemical reactions with humic material. Environ. Pollut. **70**: 183–208.

Mittleman, M.W., and Geesey, G.G. 1985. Copper binding characteristics of exopolymers. Appl. Environ. Microbiol. **49**: 846–851.

Mueller, M.E., Sanchez, D.A., and Bergman, H.L. 1991. Nature and time course of acclimation to aluminum in juvenile brook trout (*Salvelinus fontinalis*). II. Gill histology. Can. J. Fish. Aquat. Sci. **48**: 2016–2027.

Parkhurst, D.L., Thorstensen, D.C., and Plunner, L.N. 1980. A computer program for geochemical calculations. U.S. Ecol. Surv. Rep. No. USES/WRI-80-96.

Pearse, A.G. 1960. Histochemistry, theoretical and applied. Churchill, London, U.K.

Smith, R.W., and Hem, J.D. 1972. Effect of ageing on aluminium hydroxide complexes in dilute aqueous solutions. Geol. Surv. Water Supply Pap. No. 1827-D. Government Printing Office, Washington, D.C.

Thomas, J.D., Lough, A.S., and Lodge, R.W. 1975. The chemical ecology of *Biomphalaria glabrata* (Say) the snail host of *Schistosoma mansoni* (Sambon): the search for factors in media conditioned by snails which inhibit their growth and reproduction. J. Appl. Ecol. **12**: 421–436.

Truscott, R., McCrohan, C.R., Bailey, S.E.R., and White, K.N. 1995. Effect of aluminum and lead on activity in the freshwater snail *Lymnaea stagnalis*. Can. J. Fish. Aquat. Sci. **52**: 1623–1629.

Uhlinger, D.J., and White, D.C. 1983. Relationship between physiological status and formation of EPS. Appl. Environ. Microbiol. **45**: 64–70.

Wicklum, D., and Davies, R.W. 1996. The effect of chronic cadmium stress on energy acquisition and allocation in a freshwater benthic invertebrate predator. Aquat. Toxicol. **35**: 237–252.

Wilson, R.W., Bergman, H.L., and Wood, C.M. 1994. Metabolic costs and physiological consequences of acclimation to aluminum in juvenile rainbow trout (*Oncorhynchus mykiss*): 1: Acclimation specificity, resting physiology, feeding, and growth. Can. J. Fish. Aquat. Sci. **51**: 527–535.

Wolfaardt, G.M., Laureno, J.R., Headley, J.W., Roberts, R.D., and Caldwell, D.E. 1994. Microbial exopolymers provide a mechanisms for bioaccumulation of contaminants. Microb. Ecol. **27**: 279–291.

Diagnostics for multiyear tagging models with application to Atlantic striped bass (*Morone saxatilis*)

Robert J. Latour, John M. Hoenig, John E. Olney, and Kenneth H. Pollock

Abstract: Information on age- and year-specific survival can be obtained from multiyear tagging data using one of three classes of tag recovery models. Two of the model types yield information on total survival, while the third allows separation of total mortality into its fishing and "natural" components if information on the tag reporting rate is available. The performance of each class is usually assessed using goodness-of-fit tests, Akaike's information criterion, and similar measures. However, we propose that examination of model residuals is also important for the evaluation of model performance and contend that at least four types of problems are potentially detectable via patterns in residuals. Those presented in this paper include nonmixing of newly tagged animals, emigration of older animals, cohort effects associated with initial tag-induced mortality or tag shedding, and a change in the natural mortality rate. We present the diagnostic procedures by analyzing a hypothetical tagging data set and discuss the various constraints inherent to the residuals of each class of models. The diagnostic procedures are also used to evaluate striped bass tagging (*Morone saxatilis*) data from the Hudson River and Chesapeake Bay.

Résumé : Trois classes de modèles de récupération d'étiquettes permettent d'obtenir de l'information sur la survie spécifique à l'âge ou à l'année à partir de données de marquage qui s'étendent sur plusieurs années. Deux des types de modèles renseignent sur la survie totale, alors que le troisième permet de séparer la mortalité due à la pêche des composantes « naturelles » de la mortalité, si l'on connaît le taux de signalisation des étiquettes. La performance de chaque classe de modèle est généralement mesurée par des tests d'ajustement, par le critère d'information d'Akaike et d'autres mesures similaires. Nous proposons que l'examen des résidus des modèles est important dans l'évaluation de leur performance et pensons qu'au moins quatre types de problèmes peuvent être détectés par l'étude de la structure des résidus. Les problèmes signalés ici comprennent, entre autres, la répartition non aléatoire des animaux nouvellement marqués dans la population, l'émigration des animaux plus âgés, les effets sur les cohortes de la mortalité associée au marquage ou à la perte des étiquettes et le changement du taux de mortalité naturelle. L'analyse d'une série de données de marquage hypothétiques illustre les méthodes diagnostiques; on y trouve aussi une discussion des diverses contraintes associées aux résidus de chaque classe de modèles. Les méthodes diagnostiques ont aussi servi à évaluer des données de marquage du Bar rayé (*Morone saxatilis*) du fleuve Hudson et de la baie de Chesapeake.

[Traduit par la Rédaction]

Introduction

The origin and development of multiyear tagging models that allow the estimation of age- and year-specific survival and tag recovery rates have been well documented. Early work by Seber (1970), Robson and Youngs (1971), and Youngs and Robson (1975) was generalized and presented in the context of migratory bird studies by Brownie et al. (1985). Although the Brownie models were originally applied mostly to waterfowl banding data, it has been noted that the methodology can also be used to analyze fisheries tagging data. Pollock et al. (1991) and Hoenig et al. (1998*a*) showed that it is possible to convert tag recovery rates to fishing exploitation rates when information on the tag retention, tag-induced mortality, and tag reporting rate is available. They also demonstrated that it is possible to estimate instantaneous rates of fishing and natural mortality from fishing exploitation rates if additional information on the seasonal distribution of fishing intensity is known at least approximately. Hoenig et al. (1998*b*)

Received December 5, 2000. Accepted June 14, 2001. Published on the NRC Research Press Web site at http://cjfas.nrc.ca on August 9, 2001.
J16120

R.J. Latour,[1] J.M. Hoenig, and J.E. Olney. Department of Fisheries Science, Virginia Institute of Marine Science, College of William and Mary, Gloucester Point, VA 23062, U.S.A.
K.H. Pollock. Biomathematics Graduate Program, Department of Statistics, North Carolina State University, Raleigh, NC 27695, U.S.A.

[1] Corresponding author (e-mail: latour@vims.edu).

DOI: 10.1139/cjfas-58-9-1716

extended the instantaneous rates formulation to allow for nonmixing of newly tagged animals among the general population.

Case studies that involve the application of Brownie-type models to fisheries data are numerous in recent years (Larson et al. 1991; Ross et al. 1995; Smith et al. 2000). However, in many respects, the rate of theoretical development of these models has greatly exceeded the rate at which tagging studies that adhere to their assumptions and design specifications are being conducted. In practice, application of the Brownie-type models generally requires that a large portion of the data analysis process involve investigation and evaluation of biases due to potential violation of assumptions. Violation of assumptions can sometimes be attributed to the species under study (e.g., rates of survival and tag recovery may not be homogeneous within years if some animals permanently emigrate from the study area) or to the design of the tagging study (e.g., nonmixing of newly and previously tagged animals may be problematic if tagging occurs in only a few locations).

The details associated with model building and model selection have always been widely recognized as important for good statistical inference. However, until recently, model selection has received little attention in most practical situations. In the realm of tagging models, two recent advances have precipitated changes in the model selection recommendations originally made by Brownie et al. (1985). Specifically, Burnham et al. (1995) advocated placing greater reliance on Akaike's information criterion (AIC) (Akaike 1973; Burnham and Anderson 1992) and other related measures such as quasi-likelihood AIC (QAIC) (Akaike 1985) over likelihood ratio tests; Buckland et al. (1997) and Burnham and Anderson (1998) suggested estimating survival as a weighted average from multiple models instead of searching for a single "best" model.

In this paper, we build on the themes of model building and model selection by proposing a series of diagnostic procedures that can be used in conjunction with goodness-of-fit (GOF), AIC, and QAIC measures to assess the performance of a model. In essence, we suggest that the fit of a model be evaluated by critically analyzing the model residuals and that distinct patterns in those residuals will be evident if particular assumptions are violated. We include two separate tagging data sets in our discussion of the various patterns. First, a hypothetical data set is used for the purposes of explanation and demonstration of the diagnostics. That example is then followed by an exploratory analysis of Atlantic striped bass (*Morone saxatilis*) tagging data from the Hudson River, New York, and both the Maryland and Virginia portions of Chesapeake Bay.

Review of multiyear tagging models and assumptions

Brownie et al. (1985), Model 1

Tag return data are generally represented by an upper triangular matrix of tag recoveries. For example, the matrix for a study with I years of tagging and J years of recovery would be

$$(1) \qquad r = \begin{bmatrix} r_{11} & r_{12} & \cdots & r_{1J} \\ - & r_{22} & \cdots & r_{2J} \\ \vdots & \vdots & \ddots & \vdots \\ - & - & - & r_{IJ} \end{bmatrix}$$

where r_{ij} is the number of tags recovered in year j that were released in year i ($i = 1,...,I$; $j = 1,...,J$; $J \geq I$). Tagging periods do not necessarily have to be yearly intervals. However, data analysis is easiest if all periods are the same length and all tagging events are conducted at the beginning of each period. Application of the Brownie models involves constructing a matrix of expected values and comparing them with the observed data. The matrix of expected values corresponding to eq. 1 under Model 1 of the Brownie formulation would be

$$(2) \qquad E(r) = \begin{bmatrix} N_1 f_1 & N_1 S_1 f_2 & \cdots & N_1 S_1 \ldots S_{J-1} f_J \\ - & N_2 f_2 & \cdots & N_2 S_2 \ldots S_{J-1} f_J \\ \vdots & \vdots & \ddots & \vdots \\ - & - & - & N_I f_J \end{bmatrix}$$

where N_i is the number tagged in year i, f_i is the tag recovery rate in year i, and S_i is the survival rate in year i. Since the data in each row of the matrix follow a multinomial distribution, the method of maximum likelihood can be used to derive parameter estimates. Also, since all tagged cohorts are assumed to be independent, the overall likelihood function is simply the product of the individual likelihood functions corresponding to each row of the recovery matrix (Brownie et al. 1985; Hoenig et al. 1998*a*). Software packages that numerically maximize the multinomial likelihood function have been developed for application of the Brownie and other tagging models. These include programs SURVIV (White 1983) and MARK (White and Burnham 1999).

Seber (1970), Model 1*

Seber (1970) proposed a class of tag recovery models very similar to those described by Brownie et al. (1985). The only difference lies in the definition of the tag recovery rate. Specifically, Seber (1970) modeled the tag recovery rate as $f = (1 - S)r$, where r is the rate at which tags are reported from killed fish regardless of the source of mortality. Application of the time-specific parameterization of the Seber (1970) models, which we will refer to as Model 1*, again involves comparing a matrix of expected values with observed data. Program MARK can be used to numerically maximize the product likelihood function and ultimately obtain parameter estimates for this class of models.

Hoenig et al. (1998*a*), the instantaneous rates (IR) models

Hoenig et al. (1998*a*) reparameterized the Brownie models in terms of instantaneous rates of fishing mortality (F) and natural mortality (M) and showed how information on fishing effort could be used to partition total mortality into its fishing and natural components. Application of the IR models also involves constructing a matrix of expected recoveries. Under the formulation of a model that specifies time-specific fishing mortality rates and a constant natural mortality rate, the matrix of expected values corresponding to eq. 1 would be

$$(3) \qquad E(r) = \begin{bmatrix} N_1\phi\lambda u_1(F_1,M) & N_1\phi\lambda u_2(F_2,M)\mathrm{e}^{-(F_1+M)} & \dots & N_1\phi\lambda u_J(F_J,M)\mathrm{e}^{-\left(\sum\limits_{k=1}^{J-1}F_k+(J-1)M\right)} \\ - & N_2\phi\lambda u_2(F_2,M) & \dots & N_2\phi\lambda u_J(F_J,M)\mathrm{e}^{-\left(\sum\limits_{k=2}^{J-1}F_k+(J-2)M\right)} \\ \vdots & \vdots & \ddots & \vdots \\ - & - & - & N_I\phi\lambda u_J(F_J,M) \end{bmatrix}$$

where ϕ is the short-term probability of surviving the tagging process with the tag intact, λ is the tag reporting rate (ϕ and λ are considered constant over time), and $u_k(F_k,M)$ is the exploitation rate in year k (u_k is a function of F_k and M and depends on the seasonal pattern of fishing). When fishing occurs continuously throughout the year with constant intensity, the exploitation rate is (Ricker 1975)

$$u_k(F_k,M) = \frac{F_k}{F_k+M}(1-\mathrm{e}^{-F_k-M})$$

Again, the recapture data follow the multinomial distribution and parameter estimates are obtained by maximizing an overall product multinomial likelihood function. An S-PLUS-based program entitled AVOCADO (J.M. Hoenig et al., unpublished data) has been developed to fit this class of models.

Assumptions of multiyear tagging models

Application of the Brownie models requires making the following assumptions (see Pollock et al. (1991, 2001) for critical reviews of these assumptions as they relate to the analysis of fisheries tagging data): (1) the tagged sample is representative of the target population, (2) there is no tag loss, (3) survival rates are not affected by tagging, (4) the year of tag recovery is correctly reported, (5) the fate of each tagged fish is independent, and (6) all tagged fish within a cohort are subject to the same annual survival rate and recovery rate. Since development of the IR formulation was based on the traditional Brownie models, it follows that application of these models requires making those assumptions outlined above. However, two additional assumptions are also required: (7) fishing and natural mortality processes are additive and (8) the tag reporting rate is known. In theory, the tag reporting rate can be estimated from the tagging data if it is constant over time. However, Hoenig et al. (1998*a*) found that massive numbers of recaptures are necessary to get reliable estimates. Hence, external information about the tag reporting rate is often needed (e.g., from a high-reward tagging study or catch sampling program).

Residuals of Model 1 and Model 1*

Because the diagnostic procedures that we propose involve searching for patterns in the residuals, it is necessary to briefly discuss the structure of the residuals associated with Model 1 and Model 1*. A critical analysis revealed that several of the residuals associated with these models are always zero. Specifically, the relationship $E(r_{11}) = r_{11}$ always holds, regardless of the number of years of tagging and recovery. That is, the observed data and the expected value associated with the (1,1) cell are always identical, which implies that the residual for that cell is always equal to zero. Further, it can be shown that the residuals associated with the implicit "never seen again" category are also always equal to zero (analytical proof of these properties is available from R.J. Latour). Given these characteristics, the matrix of residuals derived from analyzing tag recovery data for a study with I years of tagging and J years of recovery under Model 1 or Model 1* takes the form

$$\text{resid} = \begin{bmatrix} 0.00 & (r_{12}-E_{12}) & \dots & (r_{1J}-E_{1J}) & 0.00 \\ - & (r_{22}-E_{22}) & \dots & (r_{2J}-E_{2J}) & 0.00 \\ \vdots & \vdots & \ddots & & 0.00 \\ - & - & \dots & (r_{IJ}-E_{IJ}) & 0.00 \end{bmatrix}$$

where r_{ij} is the observed number of tag recoveries and E_{ij} is the corresponding expected number of tag recoveries (the above matrix has $J + 1$ columns because a column has been added on the right to account for fish that were tagged and never recaptured). It is also true that the sum of each row and each column of the residuals matrix must equal zero. For the case when $I = J$ (i.e., the recovery matrix is square), an additional constraint that $E(r_{IJ}) = r_{IJ}$ is present. Which implies that the residual for the (I,J) cell is always equal to zero.

When searching for patterns in the residuals, the fact that those values associated with the (1,1) and the "never seen again" cells are zero essentially implies that fewer residual values are available for inspection. For example, examination of row 1 for a pattern will involve only $J - 1$ residuals rather than the $J + 1$ that are inherent to the model. Similarly, when $I = J$ and the recovery matrix is square, the Jth column only contains $J - 1$ residuals that can be scrutinized for a pattern. Having fewer available residuals is not overly problematic; rather, it just implies that conclusions about the existence of patterns may be based on fewer values. With the exception of the row sums having to be zero, the residuals given by program AVOCADO for all parameterizations of the IR models are not subject to the aforementioned constraints.

Diagnostic procedures

When fitting a multiyear tagging model, it is important to determine if any of the underlying assumptions have been violated. If model assumptions are not supported by the data, then it is likely that parameter estimates obtained from the analysis will be unreliable. One approach for evaluating model fit involves comparing the observed and expected

Table 1. Hypothetical perfect data generated by arbitrarily choosing $S_1 = 0.66$, $S_2 = 0.70$, $S_3 = 0.67$, $S_4 = 0.63$, $f_1 = 0.11$, $f_2 = 0.09$, $f_3 = 0.10$, $f_4 = 0.13$, and $f_5 = 0.11$ under Model 1 or, alternatively, $F_1 = 0.27$, $F_2 = 0.21$, $F_3 = 0.25$, $F_4 = 0.32$, $F_5 = 0.26$, $M = 0.15$, and $\phi\lambda = 0.50$ under the IR formulation with continuous fishing throughout the year.

		Number recaptured in year				
Year	Number tagged	1	2	3	4	5
1	1100	121	64	52	43	23
2	950		84	68	57	30
3	845			87	72	38
4	1020				130	68
5	990					106

Note: The recoveries were rounded to the nearest whole number. Consequently, the residuals from fits of Model 1 and an IR model are not exactly zero, but the largest residual in absolute value was less than 0.40.

numbers of tag recoveries. This comparison constitutes the basis for the formal GOF test, which is used to test the null hypothesis that the model fits the data. Although the GOF test is informative, rejection of the null hypothesis does not provide any information about why the model does not fit the data or which assumption(s) is (are) possibly in violation. Also, failure to reject the null hypothesis of a good fit does not necessarily imply that all assumptions are met. We propose a more systematic approach for assessing model performance and suggest that certain patterns in model residuals can be indicative of particular assumption violations.

Example 1. Explanation of diagnostics using a hypothetical perfect data set

For the purposes of explanation and demonstration, a hypothetical perfect data set (i.e., the observed number of tag recoveries is equal to the expected number of tag recoveries) that reflects 5 years of tagging and recovery was created (Table 1). The expected values were generated under Model 1 with arbitrarily chosen parameter values $S_1 = 0.66$, $S_2 = 0.70$, $S_3 = 0.67$, $S_4 = 0.63$, $f_1 = 0.11$, $f_2 = 0.09$, $f_3 = 0.10$, $f_4 = 0.13$, and $f_5 = 0.11$. The data in Table 1 can also be generated under the IR formulation with parameter values $F_1 = 0.27$, $F_2 = 0.21$, $F_3 = 0.25$, $F_4 = 0.32$, $F_5 = 0.26$, $M = 0.15$, and the product $\phi\lambda = 0.50$ assuming that fishing is continuous throughout the year.

To demonstrate the patterns in model residuals that develop when particular assumptions are violated, the true recovery matrix was modified to simulate five specific scenarios: nonmixing, emigration from the study area (a form of heterogeneity), tag-induced mortality, a change in the natural mortality rate, and a change in the tag reporting rate (note that this is only an assumption violation of the IR model). Since analysis of the unmodified data produces residuals exactly equal to zero, fitting a model to each of these modified data sets explicitly reveals the patterns in residuals that arise when a particular assumption is violated. When applicable, Model 1, Model 1*, and a time-specific IR model (assuming the product $\phi\lambda = 0.50$) were fit to the data using programs MARK and AVOCADO. In all cases, the patterns evident in the residuals of Model 1 were also expressed in those of Model 1*. Hence, we only refer to the residuals of Model 1 and the IR model in the discussion that follows. Also, we do not discuss in detail the change in tag reporting rate scenario, since it was not possible to detect violation of this assumption via a pattern in the residuals of the IR model.

Nonmixing

This constitutes violation of assumption 1, where newly tagged fish are not thoroughly mixed with previously tagged fish. Nonmixing of newly tagged fish among cohorts can result from a lack of dispersal (i.e., clustering) immediately after tagging. It may be that the natural migratory behavior of the species under study is such that dispersal and mixing of a released cohort of tagged fish requires more time than that defined by the tagging period of the analysis.

In their discussion of nonmixing, Hoenig et al. (1998*b*) intentionally modified the lake trout tagging data from Cayuga Lake, New York (Youngs and Robson 1975), to simulate a nonmixing scenario. A similar approach was adopted here to create a nonmixing situation with the recovery data in Table 1. Specifically, the number of recaptures along the main diagonal were multiplied by two thirds to simulate a situation where the exploitation rate of newly tagged fish is two thirds that of previously tagged fish (Table 2*a*). Since the survival rate in the year of tagging is higher, the number of recaptures in subsequent years had to be adjusted upwards. To do so, the natural mortality rate and fishing mortality rates associated with the original data were used to calculate a cohort-specific exploitation rate for each year of tagging. New fishing mortality rates, F_i^*, that reduced the exploitation rates to two thirds of their original value were identified through an iterative process. For each row of the recovery matrix, all entries to the right of the main diagonal were then multiplied by the ratio of survival rates (i.e., $\exp(F_i - F_i^*)$, where F_i is the original fishing mortality rate).

The fit of both Model 1 and the IR model to the data in Table 2*a* is extremely poor, as evident from the results of the GOF test (Model 1: $\chi^2_{6\mathrm{df}} = 18.23$, $p < 0.006$; IR model: $\chi^2_{9\mathrm{df}} = 20.2$, $p < 0.02$). However, an alternative indication that both models fit poorly is given by the consistently negative residuals on the main diagonal and positive residuals on the super diagonal of the residuals matrix for each model (Table 2*b*). It is clear that the residuals from both models possess the pattern; however, the restrictions that the (1,1) and (I,I) residuals of Model 1 are always zero forces the determination of the pattern to be based on fewer cells. Since the residuals of the IR model do not posses those same constraints, the negative pattern on the main diagonal is more obvious.

If newly tagged fish tend to remain clustered after they are released, the distribution of those individuals will be different from that of those that were tagged on prior sampling occasions. Suppose tagged fish are released in areas that are not prime fishing locations. Then newly tagged fish will be less available to fishers. A model that assumes that all tagged cohorts are well mixed cannot account for the reduced availability of newly tagged fish. Hence, the number of observed tag recoveries will be consistently smaller than the expected number of tag recoveries in the year of tagging, and a pattern of negative residuals will emerge on the main diagonal. Since nonmixing in this case causes the survival rate in the year of tagging to be abnormally high, there will be more tagged fish available for harvest in later years. A

Table 2. Tagging data from Table 1 modified to simulate incomplete mixing in the year of tagging, and residuals from fitting Model 1 and an IR model (residuals for the IR model in parentheses).

(*a*) Hypothetical data modified to simulate nonmixing in the year of tagging[a]

		Number recaptured in year				
Year	Number tagged	1	2	3	4	5
1	1100	81	70	57	48	25
2	950		56	74	61	32
3	845			58	79	41
4	1020				87	77
5	990					70

(*b*) Model residuals from fitting Model 1 and an IR model

Number recaptured in year					
1	2	3	4	5	Never seen
0.00 (–0.62)	10.43 (9.25)	1.13 (–0.74)	–3.67 (–5.79)	–7.88 (–7.94)	0.00 (5.83)
	–10.43 (–9.65)	11.70 (11.60)	3.39 (2.87)	–4.66 (–3.60)	0.00 (–1.22)
		–12.83 (–10.60)	13.50 (15.09)	–0.68 (1.86)	0.00 (–6.35)
			–13.22 (–11.21)	13.22 (16.86)	0.00(–5.66)
				0.00 (–7.17)	0.00 (7.17)

[a] Exploitation of newly tagged fish was assumed to be two thirds that of previously tagged fish (see text for a full description of the modified data).

model that assumes complete mixing among tagged cohorts cannot adjust for an increase in availability of tagged fish in later years. Hence, the expected number of tag recoveries on the super diagonal will be abnormally low and consistently positive residuals will emerge (note that if newly tagged animals are released in areas that are intensely fished, the opposite pattern will appear with the main diagonal having positive residuals).

Hoenig et al. (1998*b*) showed that the presence of nonmixing can lead to seriously biased parameter estimates if a model that assumes that all tagged cohorts are well mixed is used for data analysis. Further, they demonstrated how to model nonmixing explicitly using the IR formulation and pointed out that the Brownie models cannot be parameterized to account for this potential problem. The detection of nonmixing is certainly not trivial. Data inspection cannot be used, and it is not always possible to determine if the study design itself will facilitate proper dispersal of tagged fish (see Bertignac et al. (1999) for an example of how the availability of the tagged population changes over time). To assist with this problem, Latour et al. (2001*b*) developed a simple test for nonmixing that can be applied prior to any data analysis if tag recovery locations are known.

Emigration

This constitutes violation of assumption 6 where survival or rate of disappearance among tagged fish within a cohort is variable in a particular year. This can occur if, for example, a tagged cohort comprises several age groups and older tagged fish commence a migratory pattern that causes them to permanently leave the study area. This can also occur if the various age groups of fish within a tagged cohort exhibit different rates of chronic tag loss due to tag fouling and (or) degradation (see Henderson-Arzapalo et al. (1999) for a discussion of tag degradation) or suffer an increase in mortality due to senility.

Emigration was introduced into the recovery data in Table 1 by postulating that any changes in mortality due to movement only occur after a cohort of tagged fish has been at liberty at least 2 complete years. To simulate a scenario in which emigration increases over time, the number of recaptures in recovery years 3, 4, and 5 was reduced by 20, 30, and 40%, respectively, for the cohorts that were affected (Table 3*a*). Essentially, manipulation of the recovery data involved adjusting the number of recaptures along diagonals in the upper right corner of the matrix. Hence, the recoveries associated with cohorts 4 and 5 were unchanged in this scenario.

Unlike the nonmixing scenario, the GOF tests associated with both models suggest that the model fit is adequate (Model 1: $\chi^2_{6\text{df}} = 2.15$, $p < 0.91$; IR model: $\chi^2_{9\text{df}} = 2.35$, $p <$ 1.00). However, definitive patterns in the residuals from both models are apparent. In particular, the residuals corresponding to the diagonals in the upper right corner of the matrix (excluding the "never seen again" column) are predominately negative (Table 3*b*). Since the oldest tagged fish are generally those that are tagged at the beginning of the study and recaptured near the end of the study, it follows that there would be fewer recaptures along the diagonals in the upper right corner of the matrix if age-related emigration from the study area is evident. Consistently fewer observed than expected recoveries in that particular corner of the matrix will clearly cause the residuals to be negative. It should also be noted that the patterns discovered with the nonmixing scenario are also present in Table 3. Although this suggests that violation of assumptions 1 and 6 may be indistinguishable, the presence of negative residuals on both the main diagonal and the upper right corner of the residuals matrix is indicative of a problem that the GOF test was unable to detect. In this situation, questions should be asked about the study design (e.g., is tagging occurring in a few proximal locations or many disparate locations) and the life history of the species (e.g., at some point, will the species take on a migratory pattern that causes it to leave the study area) to identify the nature of the problem.

To account for possible senescence and emigration, a re-

Table 3. Tagging data from Table 1 modified to simulate emigration/senescence after years at liberty, and residuals from fitting Model 1 and an IR model (residuals for the IR model in parentheses).

(*a*) Hypothetical data modified to simulate emigration/senescence of older animals[a]

		Number recaptured in year				
Year	Number tagged	1	2	3	4	5
1	1100	121	64	47	35	16
2	950		84	68	51	24
3	845			87	72	34
4	1020				130	68
5	990					106

(*b*) Model residuals from fitting Model 1 and an IR model

Number recaptured in year					
1	2	3	4	5	Never seen
0.00 (–0.18)	3.67 (3.56)	1.19 (–1.55)	–1.40 (–1.82)	–1.08 (–1.02)	0.00 (1.00)
	–3.67 (–3.89)	5.22 (4.67)	–0.64 (–1.26)	–0.92 (–0.84)	0.00 (1.31)
		–4.03 (–3.15)	5.81 (6.14)	–1.78 (–1.08)	0.00 (–1.92)
			–3.77 (–2.48)	3.77(5.47)	0.00(–2.98)
				0.00 (–2.49)	0.00 (2.49)

[a] The number of recaptures in recovery years 3–5 was reduced by 20, 30, and 40% for animals at large for 3, 4, and 5 years, respectively.

cent innovation known as the "chop" option has been developed (Latour et al. 2001*a*). Essentially, the data in the upper right corner of the recovery matrix are ignored by adding those recaptures to the "never seen again" column. This will correct for some bias due to violation of assumption 6; however, subsequent parameter estimates may be less precise, since estimation is based on fewer overall data.

Tag-induced mortality

This constitutes violation of assumption 3 and an element of assumption 2, where the survival of a particular cohort of tagged fish is substantially reduced by the tagging process or tags are lost shortly after tagging. This can occur if the tagging crew changes and new members do not have much experience handling or tagging fish.

To simulate the effects of tag-induced mortality or immediate loss of tags due to tagging inexperience in year 2, the recaptures in Table 1 corresponding to cohort 2 were reduced by 20% in each recovery year (Table 4*a*). As with the emigration scenario, data analysis yielded GOF test statistics that were quite small (Model 1: $\chi^2_{6\mathrm{df}} = 0.34$, $p < 1.00$; IR model: $\chi^2_{9\mathrm{df}} = 2.28$, $p < 1.00$), which implies that both models fit the data well.

Because simulation of tag-induced mortality involved reducing the number of recaptures associated with cohort 2, it is reasonable to speculate that the effects of excessive tag-induced mortality would cause the residuals corresponding to the recaptures of cohort 2 to all be negative in both models. Although this is the case with row 2 of the IR residuals matrix, the same was not found with that of Model 1 (Table 4*b*). Since the residuals corresponding to the "never seen again" cells of Model 1 are always zero, and since each row sum of the residuals matrix must total zero, Model 1 cannot express a row pattern. Hence, from a diagnostics perspective, the constraints associated with the residuals of Model 1 render it very difficult to detect assumption violations that are cohort specific.

The residuals of the IR model are much more informative than those of Model 1 for the detection of assumption violations that are cohort specific. As with the residuals of Model 1, the row sums of the IR residuals matrix must also total zero. However, since the residuals associated with the "never seen again" cells of the IR model can assume positive or negative values, the expression of distinct row patterns is possible. This freedom makes it possible for the residuals of the recovery-year cells specific to a single cohort to all take on values of the same sign (i.e., the "never seen again" residuals can adjust for the row patterns). This is evident in Table 4*b*, since rows 1, 3, and 4 all possess positive recovery-year residuals and, as expected under the current scenario, row 2 possesses all negative residuals (row 5 is uninformative since it contains only one residual corresponding to a recovery year).

A change in the natural mortality rate

In theory, the class of models developed by Hoenig et al. (1998*a*) can be parameterized in terms of year-specific fishing and natural mortality rates. However, the desire for a parsimonious model often creates a need to assume that M is constant over time and over recruited ages in fisheries assessments. In spite of this, it is certainly possible for M to vary over time, since extreme seasonal temperatures, limitation of nutrients, and (or) pollution effects could all cause M to increase in a particular year.

To simulate the effects of a variable M, the recaptures in recovery year 5 of Table 1 were adjusted to reflect an M value of 0.45 (Table 5*a*). Even though the GOF test suggests that the fit of the IR model with a constant M is adequate ($\chi^2_{9\mathrm{df}} = 12.2$, $p < 0.20$), a distinct pattern of negative residuals is present in column 5 of the residuals matrix (Table 5*b*). The increase in M caused the number of recaptures in column 5 of Table 1 to decrease. This is not surprising, since a higher M essentially implies that fewer tagged fish will be available to fishers. By assuming that M is constant over time, the IR

Table 4. Tagging data from Table 1 modified to simulate tag-induced mortality/tag loss, and residuals from fitting Model 1 and an IR model (residuals for the IR model in parentheses).

(*a*) Hypothetical data modified to simulate tag-induced mortality/tag loss endured by cohort 2[a]

		Number recaptured in year				
Year	Number tagged	1	2	3	4	5
1	1100	121	64	52	43	23
2	950		67	55	45	24
3	845			87	72	38
4	1020				130	68
5	990					106

(*b*) Model residuals from fitting Model 1 and an IR model

Number recaptured in year					
1	2	3	4	5	Never seen
0.00 (3.35)	0.08 (7.43)	–0.08 (4.23)	–0.14 (2.99)	0.24 (1.93)	0.00 (–19.92)
	–0.08 (–9.08)	0.24 (–9.25)	–0.27 (–8.81)	0.12 (–4.34)	0.00 (31.48)
		–0.06 (4.29)	0.03 (2.72)	0.03 (1.52)	0.00 (–8.54)
			0.38 (3.04)	–0.39 (1.14)	0.00(–4.18)
				0.00 (–0.30)	0.00 (0.30)

[a] The number of recaptures corresponding to cohort 2 in recovery years 2–5 was each reduced by 20%.

Table 5. Tagging data from Table 1 modified to simulate a change in the natural mortality rate *M*, and residuals from fitting an IR model that assumes that *M* is constant.

(*a*) Hypothetical data modified to simulate a change in *M*[a]

		Number recaptured in year				
Year	Number tagged	1	2	3	4	5
1	1100	121	64	52	43	6
2	950		84	68	57	11
3	845			87	72	18
4	1020				130	44
5	990					92

(*b*) Model residuals from fitting Model 1 and an IR model

Number recaptured in year					
1	2	3	4	5	Never seen
2.02	3.43	4.86	6.41	–5.16	–11.56
	–2.05	1.03	5.01	–4.85	0.87
		–6.24	–0.38	–4.07	10.68
			–14.64	–0.10	14.74
				13.98	–13.98

[a] The value of *M* was increased from 0.15 to 0.45 in year 5.

model cannot account for the systematically fewer recaptures in recovery year 5, and the residuals associated with that recovery year are consistently negative (in cases where *M* decreased in a particular year, a column pattern of positive residuals would emerge). It should be noted that the change in *M* precipitated the development of row patterns. Specifically, the residuals in row 1 are consistently positive and the residuals in rows 3 and 4 are strictly negative.

Subtle changes in *M* do not readily create column patterns in the matrix of residuals. Simulations where *M* was only slightly changed in a particular year failed to produce consistent patterns of negative or positive residuals. Since the detection of a change in *M* is not trivial, we suggest that year-specific auxiliary data (e.g., prey indices, water temperature, etc.) be used in combination with examination of residuals to determine the likelihood of a change in *M*.

Sensitivity of diagnostics under the IR formulation

Because the partition of total mortality into its fishing and natural components is sensitive to the estimate of $\phi\lambda$ (Latour et al. 2001*a*), we varied the value of $\phi\lambda$ to determine if the aforementioned patterns in residuals were apparent under conditions when the true and assumed values of $\phi\lambda$ were substantially different. Since the hypothetical data were generated with a value of 0.50 for $\phi\lambda$, we reanalyzed the data associated with the nonmixing, emigration, tag-induced mor-

Table 6. Tag recovery data for striped bass >711 mm total length that were tagged in the Rappahannock River, Virginia, and residuals from fitting Model 1*.

(*a*) Release and recovery data for striped bass in the Rappahannock River, Virginia

Year	Number tagged	Number recaptured in year 1988	1989	1990	1991	1992	1993	1994	1995	1996	1997	1998	1999
1988	56	7	7	4	1	0	2	0	0	0	1	0	0
1989	101		4	4	3	3	0	2	0	0	0	1	0
1990	300			26	9	15	2	3	7	1	0	2	1
1991	390				41	24	16	11	3	2	2	1	2
1992	40					4	3	2	2	0	0	0	1
1993	212						22	18	7	5	6	0	0
1994	123							9	7	5	1	2	0
1995	209								28	10	8	3	3
1996	66									1	3	1	0
1997	212										15	13	8
1998	158											24	13
1999	162												16

(*b*) Residuals from fitting Model 1*

Number recaptured in year 1988	1989	1990	1991	1992	1993	1994	1995	1996	1997	1998	1999	Never seen
0.00	3.41	1.32	–0.46	0.00	1.42	0.00	0.00	0.00	0.91	0.00	0.00	–4.55
	–3.41	–1.53	0.00	0.72	0.00	1.08	0.00	0.00	0.00	0.88	0.00	4.55
		0.21	–5.01	4.33	–3.59	–1.30	4.16	–0.09	0.00	1.45	0.67	0.00
			5.47	–3.06	1.81	0.09	–4.16	–0.75	–0.12	–0.41	1.17	0.00
				–0.89	0.44	0.03	0.70	0.00	0.00	0.00	0.85	0.00
					1.12	1.93	–3.59	0.947	2.88	0.00	0.00	0.00
						–1.39	0.15	2.38	–1.02	0.66	0.00	0.00
							3.66	0.69	0.83	–1.77	0.19	–3.60
								–2.33	0.44	–0.70	0.00	3.60
									–2.51	1.37	1.15	0.00
										0.92	–0.59	–0.32
											–0.32	0.32

tality, and variable *M* scenarios using values of $\phi\lambda$ that ranged from 0.30 to 0.80.

For all values of $\phi\lambda$ considered, the patterns in residuals associated with each scenario remained evident. In fact, for the nonmixing and emigration cases, a cell-by-cell comparison of the matrices of residuals showed that the signs of all residuals remained unchanged. The same cell-by-cell preservation of positive and negative residuals was predominately evident when the tag-induced mortality and variable *M* residuals matrices were compared. For the tag-induced mortality scenario, the only differences were with row 5 of the $\phi\lambda = 0.60$, 0.70, and 0.80 scenarios. In all three instances, the residual associated with recovery year 5 was negative and the corresponding "never seen again" residual was positive (the exact opposite pattern is present in row 5 of the $\phi\lambda = 0.50$ residuals matrix). For the variable *M* scenario, the only differences were when $\phi\lambda = 0.30$ and 0.40, since three of the five residuals in column 5 were negative instead of four out of five.

Example 2. Exploration of Atlantic striped bass tagging data

The Atlantic striped bass has historically supported several important recreational and commercial fisheries along the eastern coastline. During the 1960s and 1970s, overfishing, pollution, and reduction of spawning habitat precipitated steady declines in the abundance of virtually all stocks in the Atlantic. In response to the documented decline, the Atlantic States Marine Fisheries Commission sponsored the development of an interstate fisheries management plan that took effect during the mid-1980s. The fisheries management plan called for the establishment of a cooperative tagging program (which currently involves 15 state and federal agencies) so that information on migration, relative contribution to the coastal population, and annual survival of Atlantic striped bass stocks could be evaluated. At present, the agencies participating in the cooperative tagging program continue to tag thousands of striped bass each year.

The current protocol to analyze the tagging data, as established by the Atlantic States Marine Fisheries Commission Striped Bass Technical Committee, involves deriving survival estimates from a suite of Seber (1970) models. Tag recoveries from striped bass that were >711 mm total length at the time of tagging are analyzed because they constitute the migratory population and are believed to be fully recruited to the coastal fishery. The analysis consists of four steps. First, a set of biologically reasonable candidate models is identified prior to the data analysis. These models are then fit to the tagging data, and AIC and GOF diagnostics are used to evaluate their fit (Burnham et al. 1995). The overall

Table 7. Residuals from fitting Model 1* to striped bass tagging data from the Maryland portion of Chesapeake Bay (1988–1997), and residuals from fitting Model 1* to striped bass tagging data from the Hudson River, New York (1988–1997).

(*a*) Residuals from fitting Model 1* to the Maryland striped bass tagging data

Number recaptured in year										
1988	1989	1990	1991	1992	1993	1994	1995	1996	1997	Never seen
0.00	3.17	–1.08	5.97	0.35	–0.29	0.53	0.00	0.00	0.00	–6.12
	–3.17	0.84	1.71	–0.49	–0.07	0.39	2.65	0.73	0.00	–1.76
		0.24	–6.00	0.46	–0.99	–1.84	0.55	0.00	1.65	7.79
			–1.68	–1.11	5.13	–2.90	0.01	–1.32	1.88	0.00
				0.78	–0.61	–2.80	–0.17	0.33	2.46	0.00
					–3.17	5.45	2.62	–6.69	1.79	0.00
						1.17	–1.02	0.26	–0.41	0.00
							–3.32	0.15	3.17	0.00
								9.11	–9.11	0.00
									0.00	0.00

(*b*) Residuals from fitting Model 1* to the Hudson River striped bass tagging data

Number recaptured in year										
1988	1989	1990	1991	1992	1993	1994	1995	1996	1997	Never seen
0.00	1.52	–1.30	1.79	2.39	0.09	2.42	0.00	1.63	1.02	–7.68
	–1.52	–1.02	–0.59	–4.63	–0.52	2.06	2.12	0.00	0.00	7.68
		2.32	–0.51	4.25	0.31	–2.70	–0.64	–3.11	0.08	0.00
			–0.69	0.15	–3.63	1.23	1.85	1.79	–0.70	0.00
				–2.16	1.07	0.16	–0.84	–1.92	3.70	0.00
					2.68	1.20	–6.21	–0.11	2.44	0.00
						–4.38	–1.22	1.79	3.81	0.00
							6.82	–3.40	–3.42	0.00
								5.43	–5.43	0.00
									0.00	0.00

estimates of survival are then calculated as a weighted average of survival from the best-fitting models, where the weight is related to the AIC value (i.e., the better the AIC, the higher the weight) (Buckland et al. 1997; Burnham and Anderson 1998; Smith et al. 2000).

We examined the residuals associated with the application of Model 1* to striped bass tagging data from the Hudson River, New York, the Maryland portion of Chesapeake Bay (see Smith et al. (2000) for the data from those regions) and the Rappahannock River, Virginia (Table 6*a*), to determine if model assumptions were in violation. In each of the three residuals matrices, the symptoms associated with a nonmixing or emigration problem were not present (Tables 6*b* and 7). The lack of nonmixing with the Rappahannock River data is consistent with the findings of Latour et al. (2001*b*), who performed a spatial analysis of the tag recoveries. Given the migratory behavior of striped bass >711 mm total length, this result is not surprising and it seems likely that a similar spatial analysis of the New York and Maryland data would also show that lack of mixing is not problematic. Emigration should also not be problematic, since the recovery areas for all three analyses encompass the entire eastern seaboard.

Inspection of the "never seen again" columns of the three residuals matrices does suggest that there are cohort-specific problems with the application of Model 1*. For the Virginia data, the residuals associated with the "never seen again" category of rows 1, 2, 8, 9, 11, and 12 were nonzero (Table 6*b*). A more detailed examination of the recovery data for the cohorts tagged in 1988 and 1989 showed that in 1989, there were approximately twice as many fish tagged as in 1988, but comparatively, only about half the number of tag recoveries were observed each year from the 1989 cohort. Since the data in rows 1 and 2 of the recovery matrix are needed for the estimation of S_1, it seemed likely that the problem would also be apparent with the estimate of S_1. Although there was nothing unusual about the estimate of S_1 from Model 1* ($S_1 = 0.88$), application of Model 1 (via program MARK) to the data in Table 6*a* yielded an estimate of exactly 1.00 for S_1. This estimate resulted because the program was forced to impose the constraint that all survival estimates must be between 0.00 and 1.00 during the estimation process.

Hand calculation of the estimate for S_1 under Model 1 from the analytical formula of the maximum likelihood estimate given by Brownie et al. (1985) yielded a value of 1.59 for S_1. (If the identity link in program MARK is used with Model 1, then hand calculations are not needed because MARK will provide the appropriate maximum likelihood estimates and residuals for situations where inadmissible survival estimates arise. However, the identity link does not work with Model 1* because the term $(1 - S)$ appears directly in the likelihood, and negative probability values are generated when inadmissible survival estimates arise.) Clearly, a survival probability cannot exceed 1.00, and the fact that the data yield an inadmissible value under Model 1 suggests that there is a problem with the 1989 cohort. Since a hand calculation of S_8 yielded an estimate of 1.68, it appears that there is also a problem with the 1996 cohort. However, in 1996, only 66 fish were tagged, and only a total of five recoveries were tabulated over the 4 years that the cohort was at liberty. Hence, the noted problem with S_8 and the 1996 cohort may be a result of sparse data. It is unclear why the "never seen again" residuals in rows 11 and 12 are nonzero, since hand calculation of S_{11} yielded a value of 0.89. Perhaps the constraints imposed for the estimation of S_1 and S_8 caused problems with the estimation of other parameters (it is unclear how the estimation process is affected when boundary values are reached).

For the Maryland data, the residuals associated with the "never seen again" category of rows 1–3 were nonzero (Table 7*a*). The estimates of S_1 and S_2 from Model 1* were high ($S_1 = 0.96$ and $S_2 = 0.94$), but given the fact that striped bass in Maryland were under moratorium until 1990, high rates of survival for 1988 and 1989 should be expected. However, application of Model 1 yielded estimates of exactly 1.00 for S_1 and S_2 and hand calculations of S_1 and S_2 yielded values of 1.10 and 1.11, respectively. These results are nonsensical and they suggest that there may be problems with the 1989 and 1990 cohorts. This same trend was also apparent with the New York data, since the "never seen again" residuals associated with rows 1 and 2 were nonzero (Table 7*b*). Again, the estimate of S_1 from Model 1* was high ($S_1 = 0.90$), but the hand calculated value under Model 1 was 1.06, which again suggests that there may be a problem with the 1989 cohort.

Examination of the residuals matrices (not shown here) associated with application of an IR model to each of the recovery matrices confirmed the noted cohort-specific problems. For the Virginia data, rows 2 and 8 of its residuals matrix revealed negative residuals for eight out of 11 and four out of four values, respectively. For the New York data, nine out of 10 residuals in row 1 were positive, while seven out of nine residuals were negative in row 2. For the Maryland data, row 3 of the residuals matrix showed a distinct pattern, since seven out of eight values were negative. Patterns in rows 1 and 2 were slightly less apparent, since seven out of 10 and five out of nine residuals were positive, respectively.

Discussion

We have shown that specific patterns in the residuals of multiyear tagging models arise when particular assumptions are violated. In particular, patterns along the main and super diagonals are evident when nonmixing is problematic, and patterns of consistently negative residuals are present in the upper right corner of the residuals matrix when the survival or rate of disappearance among tagged fish within a cohort is variable because of emigration. Although the patterns in residuals associated with emigration seem to be indistinguishable from those of nonmixing, the two phenomena are generally separable if the sampling design and the life history of the species under study are critically examined to determine which is more likely to be evident in the study. The presence of distinctive row patterns in the residuals matrix is indicative of short-term tag-induced/handling mortality and tag loss. A change in the natural mortality rate can cause a column effect of the IR model residuals.

An inspection of the residuals matrix for patterns should accompany the more traditional measures of model fit (e.g., AIC and GOF) as fundamental parts of evaluating the performance of multiyear tagging models. In addition to considering multiple model parameterizations, the analysis of tagging data should also involve all available model types (i.e., Brownie et al. (1985) and Hoenig et al. (1998*a*, 1998*b*) models) regardless of the goal of the study or the desired parameters estimates. Application of a suite of model types to tagging data increases the amount of information that can be used for the evaluation of assumption violation. The value of this approach is particularly exemplified by the fact that the residuals of the IR models do not possess the constraints associated with those of Model 1 and Model 1*. The symptoms of nonmixing are certainly more definitively expressed in the residuals of an IR model, since the values in the (1,1) and (I,I) cells (when $I = J$) need not be zero. Also, the freedom of nonzero "never seen again" residuals allows the IR models to accurately capture the row patterns in the residuals matrix that emerge when cohort-specific problems are present.

The conclusions about the existence of patterns in model residuals for the simulated scenarios were based strictly on the sign of the value. It may also be possible to detect the same patterns and information pertaining to the relative magnitude of each residual by examining standardized residuals. For multiyear tagging models, a standardized residual is defined as $d_{ij} = (O_{ij} - E_{ij})\big/\sqrt{E_{ij}(1 - E_{ij}/N_{ij})}$, where O_{ij} and E_{ij} represent the observed and fitted values, respectively. Consideration of the relative magnitude of model residuals can assist with the detection of outliers. If a model fits a particular data set well, then the standardized residuals should only rarely be greater than 2–3. Many large standardized residuals is indicative of overdispersion. If the variance in the data is greater than the theoretical variance predicted by the multinominal distribution, then overdispersion is present. This implies that the calculated standard errors generated from software packages for the analysis of tagging data (e.g., programs SURVIV and MARK) are generally overly optimistic about precision.

Examination of the residuals associated with the analysis of each striped bass recovery matrix revealed the presence of cohort-specific problems. The nonzero "never seen again" residuals in rows 1 and 2 of each residuals matrix were caused by the same counterintuitive trend. For each stock, approximately the same or fewer tags were recovered from the 1989 cohort than from the 1988 cohort even though significantly more fish were tagged in 1989. Based on the data, it appears that the number of striped bass effectively tagged in each state is substantially different from the actual number tagged in 1989. The most obvious explanation is short-term tagging mortality and (or) tag loss, and since the striped bass tagging program began in 1988, it is possible that inexperience with the sampling and tagging procedures (which would likely be present for all state agencies) allowed these phenomena to be present in the study. An alternative explanation is that perhaps the natural mortality rate of the fishes tagged in 1989 increased substantially due to some type of large-scale cohort-specific environmental or biological effect.

Acknowledgements

We gratefully acknowledge the continuing efforts of the Anadromous Fishes Research Program of the Virginia Institute of Marine Science who tag and release striped bass in Virginia's rivers. We thank Carl Walters and two anonymous reviewers for their helpful comments. This research was funded by the Wallop-Breaux Program of the U.S. Fish and Wildlife Service through the Recreational Fishing Advisory Board of the Virginia Marine Resources Commission (grant No. F-77-R-12). K.H. Pollock was supported by the South Carolina Department of Natural Resources (grant No. NA77FL0290). This is Virginia Institute of Marine Science contribution No. 2383.

References

Akaike, H. 1973. Information theory as an extension of the maximum likelihood principle. *In* Second International Symposium on Information Theory. *Edited by* B.N. Petrov and F. Csaki. Akademiai Kiado, Budapest, Hungary.

Akaike, H. 1985. Prediction and entropy. *In* A celebration of statistics. *Edited by* A.C. Atkinson and S.E. Fienberg. Springer, New York.

Bertignac, M., Hampton, J., and Coan, A.L., Jr. 1999. Estimates of exploitation rates for north Pacific albacore, *Thunnus alalunga*, from tagging data. Fish. Bull. U.S. **97**: 421–433.

Brownie, C., Anderson, D.R., Burhnam, K.P., and Robson, D.S. 1985. Statistical inference from band recovery data: a handbook. 2nd ed. U.S. Fish Wildl. Serv. Resour. Publ. No. 156.

Buckland, S.T., Burnham, K.P., and Augustin, N.H. 1997. Model selection: an integral part of inference. Biometrics, **53**: 603–618.

Burnham, K.P., and Anderson, D.R. 1992. Data-based selection of an appropriate biological model: the key to modern data analysis. *In* Wildlife 2001: populations. *Edited by* D.R. McCullough and R.H. Barrett. Elsevier Science Publishers, London, U.K.

Burnham, K.P., and Anderson, D.R. 1998. Model selection and inference: a practical information theoretical approach. Springer-Verlag, New York.

Burnham, K.P., White, G.C., and Anderson, D.R. 1995. Model selection strategy in the analysis of capture–recapture data. Biometrics, **51**: 888–898.

Henderson-Arzapalo, A., Rago, P., Skjeveland, J., Mangold, M., Washington, P., Howe, J., and King, T. 1999. An evaluation of six internal anchor tags for tagging juvenile striped bass. N. Am. J. Fish. Manage. **19**: 482–493.

Hoenig, J.M., Barrowman, N.J., Hearn, W.S., and Pollock, K.H. 1998*a*. Multiyear tagging studies incorporating fishing effort data. Can. J. Fish. Aquat. Sci. **55**: 1466–1476.

Hoenig, J.M., Barrowman, N.J., Pollock, K.H., Brooks, E.N., Hearn, W.S., and Polacheck, T. 1998*b*. Models for tagging data that allow for incomplete mixing of newly tagged animals. Can. J. Fish. Aquat. Sci. **55**: 1477–1483.

Larson, S.C., Saul, B., and Schleiger, S. 1991. Exploitation and survival of black crappies in three Georgia reservoirs. N. Am. J. Fish. Manage. **11**:604–613.

Latour, R.J., Pollock, K.H., Wenner, C.A., and Hoenig, J.M. 2001*a*. Estimates of fishing and natural mortality for red drum *Sciaenops ocellatus* in South Carolina waters. N. Am. J. Fish. Manage. In press.

Latour, R.J., Hoenig, J.M., Olney, J.E., and Pollock, K.H. 2001*b*. A simple test for nonmixing in multi-year tagging studies: application to striped bass tagged in the Rappahannock River. Trans. Am. Fish. Soc. **130**: 848–856.

Pollock, K.H., Hoenig, J.M., and Jones, C.M. 1991. Estimation of fishing and natural mortality when a tagging study is combined with a creel survey or port sampling. Am. Fish. Soc. Symp. **12**: 423–434.

Pollock, K.H., Hoenig, J.M., Hearn, W.S., and Calingaert, B. 2001. Tag reporting rate estimation: an evaluation of the reward tagging method. N. Am. J. Fish. Manage. In press.

Ricker, W.E. 1975. Computation and interpretation of biological statistics of fish populations. Bull. Fish. Res. Board Can. No. 191.

Robson, D.S., and Youngs, W.D. 1971. Statistical analysis of reported tag recaptures in the harvest from an exploited population. Biometrics Unit Mimeo. Ser. Pap. No. BU-369-M. Cornell University, Ithaca, N.Y.

Ross, J.L., Stevens, T.M., and Vaughan, D.S. 1995. Age, growth, mortality, and reproductive biology of red drums in North Carolina waters. Trans. Am. Fish. Soc. **124**: 137–154.

Seber, G.A.F. 1970. Estimating time-specific survival and reporting rates for adult birds from band returns. Biometrika, **57**: 313–318.

Smith, D.R., Burnham, K.P., Kahn, D.M., He, X., Goshorn, C.J., Hattala, K.A., and Kahnle, A.W. 2000. Bias in survival estimates from tag-recovery models where catch-and-release is common, with an example from Atlantic striped bass (*Morone saxatilis*). Can. J. Fish. Aquat. Sci. **57**: 886–897.

White, G.C. 1983. Numerical estimation of survival rates from band-recovery and biotelemetry data. J. Wildl. Manage. **47**: 716–728.

White, G.C., and Burnham, K.P. 1999. Program MARK — survival estimation from populations of marked animals. Bird Study, **46**: 120–138.

Youngs, W.D., and Robson, D.S. 1975. Estimating survival rates from tag returns: model tests and sample size denominations. J. Fish. Res. Board Can. **32**: 2365–2371.

Biological implications of empirical models of winter oxygen depletion

Marianne E. Meding and Leland J. Jackson

Abstract: We compiled data for 23 North American temperate zone lakes to assess three alternative winter O_2 depletion models for estimating O_2 dynamics from freezing to thawing. Dissolved O_2 concentrations were constant or declined slightly for an average of 40 days after freezing and then declined rapidly. Once O_2 concentrations reached 1–3 $mg \cdot L^{-1}$, consumption slowed. No model that we fit captured O_2 dynamics shortly after freezing. The best fit was a one-pool exponential decay model after one to four initial data were removed. Photosynthesis and freeze-out estimates suggest that O_2 inputs are more important in shallow than in deep lakes. Oxygen decay rates (k) correlated with morphometry in shallow lakes and chlorophyll *a*, Secchi depth, and the sediment surface area to volume ratio in deep lakes. We hypothesize that the failure of chlorophyll *a* to correlate with k in shallow lakes is because macrophytes are the primary source of decaying organic matter but have not been included in assessments of winter O_2 depletion. Thus, some processes in deep lakes cannot simply be scaled to smaller scales in shallow lakes.

Résumé : La compilation de données provenant de 23 lacs tempérés d'Amérique du Nord a permis d'évaluer trois modèles différents d'estimation de la dynamique de l'épuisement de l'oxygène à partir de l'engel jusqu'au dégel. Les concentrations d'oxygène dissous restent stables ou déclinent légèrement durant les 40 premiers jours après l'engel en moyenne, pour ensuite décliner rapidement. Lorsque les concentrations d'oxygène atteignent 1–3 $mg \cdot L^{-1}$, la consommation d'oxygène ralentit. Aucun des modèles utilisés ne décrit adéquatement la dynamique de l'oxygène peu après l'engel. Le modèle le mieux ajusté est un modèle exponentiel de décomposition à un seul pool, duquel on a enlevé de une à quatre des premières données. Les estimations de photosynthèse et d'épuisement d'oxygène indiquent que les apports d'oxygène sont plus importants dans les lacs peu profonds que dans les lacs profonds. Les taux de disparition de l'oxygène (k) sont en corrélation avec la morphométrie dans les lacs peu profonds et avec la chlorophylle *a*, la profondeur de Secchi et le rapport surface des sédiments : volume du lac dans les lacs profonds. Le fait qu'il n'y ait pas de corrélation entre la chlorophylle *a* et k dans les lacs peu profonds, est dû, croyons-nous, à ce que les macrophytes sont la source principale de matière organique en décomposition, mais qu'ils n'ont pas été inclus dans l'évaluation de l'épuisement de l'oxygène pendant l'hiver. Ainsi, certains processus en opération dans les grands lacs ne peuvent pas être appliqués aux lacs peu profonds par simple réduction d'échelle.

[Traduit par la Rédaction]

Introduction

The dissolved O_2 concentration of lake waters affects the distribution and diversity of organisms (Tonn and Magnuson 1982; Rahel 1984; Nürnberg 1995). Anoxia alters lake structure and function. Structure is determined by physical and biological attributes such as morphometry, climate, and the food web present. Function relates to rates of processes in the system.

Anoxia and subsequent fishkills have serious implications for structure and function in lakes. When winter O_2 concentrations fall below minimum tolerance levels for fish, they suffocate (Greenbank 1945; Barica and Mathias 1979). The extent and frequency of fishkills affect the structure of fish assemblages (Tonn and Magnuson 1982; Rahel 1984) with implications for lower trophic levels and thus food web structure (Carpenter et al. 1985, 1992; McQueen et al. 1986). Anoxia influences the types and rates of chemical processes (e.g., sulfate reduction, denitrification, and methanogenesis) by causing a shift from aerobic to anaerobic respiration with effects on biological processes such as nutrient cycling and retention. Release of toxic compounds such as methane and hydrogen sulfide further endangers organisms. Changes in lake metabolism influence lake productivity and water quality. For example, rapid nutrient enrichment increases O_2 depletion rates and leads to decreased water quality (Snodgrass and Ng 1985; Lind 1987; Gelda and Auer 1996). Consequently, there is a desire to understand the processes that control O_2 dynamics and a need to predict anoxia and fishkills if management goals are to sustain productive fisheries and maintain ecosystem stability.

Oxygen dynamics in lakes can be modeled as a mass balance. The rate of change in O_2 concentration, $d[O_2]/dt$, at any given time is a balance between O_2 inputs and losses. While ice free, photosynthesis and atmospheric exchange, largely via wind aeration, are the primary inputs of O_2 into lakes. Losses are due to organismal respiration plus chemical oxidation of reduced materials. During winter, temperate zone lakes are sealed by ice from the atmosphere for approx-

Received March 22, 2001. Accepted June 6, 2001. Published on the NRC Research Press Web site at http://cjfas.nrc.ca on August 9, 2001.
J16276

M.E. Meding and L.J. Jackson.[1] Ecology Division, Department of Biological Sciences, University of Calgary, 2500 University Drive NW, Calgary, AB T2N 1N4, Canada.

[1] Corresponding author (e-mail: ljackson@ucalgary.ca).

DOI: 10.1139/cjfas-58-9-1727

imately 4–5 months. If snow cover persists, O_2 inputs via photosynthesis become minimal (Schindler and Nighswander 1970; Prowse and Stephenson 1986). Hence, after freezing, the mass of O_2 remaining must be sufficient to meet O_2 demands for the duration of ice cover. Whether or not a lake goes anoxic while ice covered depends on the initial mass of O_2 stored at freezing, the amount and rate of organic matter (OM) decomposition, and the length of snow and ice cover. If anoxia occurs and persists for weeks, fish die.

Rates of O_2 decay depend on the concentration of substrate available for decomposition (Greenbank 1945) plus environmental factors such as temperature. One way to model O_2 depletion is to treat it as a linear process. This assumes that O_2 depletion is a first-order process where decay depends only on the concentration of the substrate:

$$(1) \qquad O_2 = A - k(t)$$

where A is the initial O_2 concentration at freezing (grams per square metre), k is the O_2 depletion rate (grams per square metre per day), and t is days after freezing. Alternatively, because many biological processes in nature are nonlinear, the following first-order exponential decay model might be more appropriate:

$$(2) \qquad O_2 = Ae^{-kt}$$

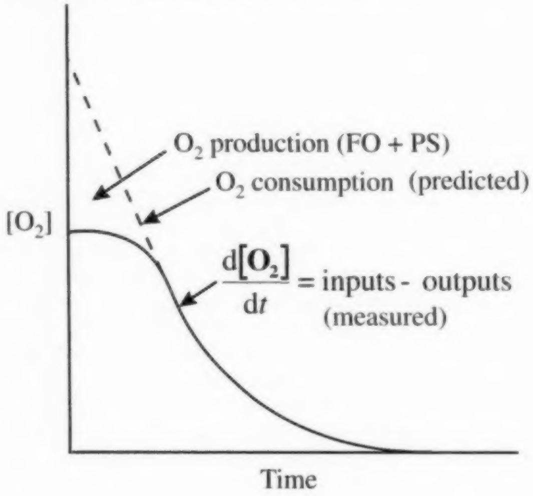
where O_2 and A are as for eq. 1 and "e" is the base of the natural logarithm. This model assumes that there is effectively a single pool of OM and that the mass of O_2 consumed decreases proportionately over time. Alternatively, more than one pool of OM could exist, with decomposition of each pool occurring nonlinearly and at different rates. A non-linear two-pool model would take the form

$$(3) \qquad O_2 = Be^{-k_1 t} + Ce^{-k_2 t}$$

where the first term (k_1) expresses the decay of one pool, the second term (k_2) describes the decay of a second pool, and B and C add up to the initial O_2 concentration. Two pools could exist if, for example, OM consisted of a rapidly decomposing labile pool and a pool of more refractive, complex compounds that take longer to decompose. Decomposition of the fast pool would dominate the initial O_2 depletion dynamics, but eventually, this pool of OM would become small enough that the decomposition of the slow pool would govern the O_2 dynamics. The resultant O_2 depletion curve would be the sum of the decay of the fast and slow pools of OM.

In holomictic lakes, the water is generally 100% saturated, with approximately 13–15 mg $O_2 \cdot L^{-1}$ after fall overturn. Once ice cover develops, and snow cover persists, O_2 concentrations decline rapidly (Barica and Mathias 1979). This rapid decline can be attributed partly to an increase in OM made available for decomposition through sediment resuspension during fall overturn and from decay of senescing OM at the end of the growing season. Once concentrations reach 1–3 mg $O_2 \cdot L^{-1}$, rates of aerobic decomposition are governed by transport of O_2 to the substrate (Burns 1995). Oxygen consumption slows, evidenced by a flattening of the O_2–time curve, until the lake is recharged with O_2 (Fig. 1). Oxygen decay has been traditionally modeled as a first-order linear process determined by regressing areal or volumetric measures of O_2 on time (eq. 1) (Barica and Mathias 1979; Babin and Prepas 1985; Cornett 1989), and typically, only the linear portion of the complete time course has been used to estimate decay rates. This method excludes the tails of the curve, ignoring initial and latter stages of O_2 depletion, which occur more slowly. Babin and Prepas (1985) acknowledged nonlinearity in O_2 depletion over time and characterized O_2 depletion by fitting two linear lines to their data, suggesting the existence of two separate pools of OM that were decomposing at different rates. Jackson and Lasenby (1982) also recognized nonlinearity in O_2 depletion over time, but only in individual lake strata of 1 m depths. They calculated individual stratum O_2 depletion rates by log transforming O_2 concentrations and then calculating depletion rates via linear regression.

Fig. 1. Conceptual O_2 depletion curve. During the initial period after freezing, inputs of O_2 via FO (expulsion of O_2 when water freezes) and PS balance losses due to respiration. The broken line indicates the extent of O_2 consumption during the initial phase by forcing the decay line of best fit back to time 0. The area between the predicted and measured curves indicates the extent of O_2 inputs via FO and PS (method I). For method II, we assumed that once inputs stop, O_2 was consumed at the rate determined by the one-pool exponential decay model. The initial O_2 concentration remained constant during the initial phase in the O_2–time course because inputs balance losses. Given the rate of O_2 consumption, the initial O_2 concentration, and the time over which inputs occur, the amount of O_2 consumed was determined and should equal O_2 inputs via PS and FO.


O_2 production (FO + PS)
O_2 consumption (predicted)
$[O_2]$
$\frac{d[O_2]}{dt}$ = inputs - outputs
(measured)
Time

Here, we evaluate the ability of models 1–3 to describe O_2 depletion from freezing to thawing in temperate zone lakes. Models 2 and 3 are contrasted with traditional linear O_2 depletion models (model 1). We then construct a simple process-based model of O_2 depletion to partition changes in O_2 concentrations among input and output processes. Finally, we determine which lake and watershed characteristics correlate with O_2 depletion rates from our model of best fit.

Materials and methods

Winter dissolved O_2 concentrations and lake and watershed variables were compiled for 23 lakes located in the prairies (Alberta

Table 1. Summary statistics including region, sample year, mean depth (z_{mean}), surface area (SA), and maximum summer Chl *a* for 23 temperate zone lakes culled from the literature.

Region	Lake	Year	z_{mean} (m)	SA (ha)	Chl *a* (μg·L^{-1})
Manitoba[a]	019	1975	3.39	28.7	38.00
	200	1976	2.97	9.87	35.00
	255	1974–1976	1.75	3.44	17.67
	318	1974–1976	1.57	24.1	50.00
	587	1974–1976	3.45	6.88	80.00
	675	1974–1976	2.7	9.12	24.67
	721	1974–1976	1.61	7.08	17.33
	882	1974–1976	2.1	5.97	157.50
	885	1974–1976	1.84	2.28	254.00
	Nora	1975	4.2	27.3	22.00
Alberta[b,c]	Stephenson's Pond (STP)	1984	1.54	1.93	—
	Peterson's Meadow Pond (PMP)	1984	2.23	2.9	—
	Lessard (Les)	1982	3.9	321	97.00
	Nakamun (Nak)	1982	4.5	354	135.00
Wisconsin[d]	Big Muskullenge (BM)	1981–1982	7.5	396.3	4.00
	Crystal Bog (CB)	1981–1982	1.7	0.5	17.30
	Crystal Lake (CR)	1981–1982	10.4	36.7	2.80
	Sparkling (SP)	1981–1982	10.9	81.4	3.90
	Trout Bog (TB)	1981–1982	5.6	1.1	28.30
	Trout Lake (TR)	1981–1982	17.1	769.8	3.20
Ontario[e,f]	Coon	1975	2.9	53	—
	ELA 239	1968	10.5	56.1	1.50
	ELA 240	1968	6.1	44.1	2.00

Note: Maximum summer Chl *a* is averaged across sample years 1974–1976 for Manitoba lakes and 1981–1982 for LTER lakes.
[a] Barica and Mathias (1979).
[b] STP and PMP taken from Baird et al. (1987).
[c] Les and Nak taken from Crosby et al. (1990) and Babin and Prepas (1985).
[d] LTER database at http:/www.limnosun.limnology.wisc.edu/
[e] Coon taken from Prowse and Stephenson (1986).
[f] ELA 239 and 240 taken from Schindler (1971) and Brunskill and Schindler (1971).

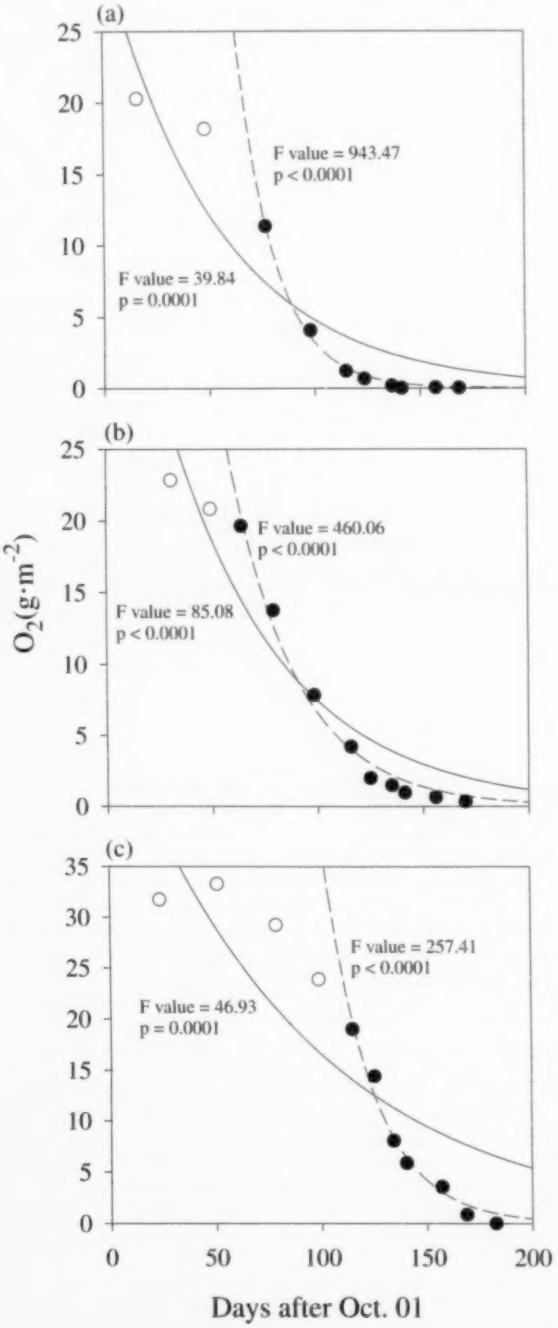
and Manitoba), Ontario, and Wisconsin (Table 1). Up to 3 years of data were available for some lakes. If not reported (Experimental Lakes Area (ELA) and Long Term Ecological Research (LTER) lakes), we calculated winter O_2 mass using O_2 concentration depth profiles and lake bathymetry. The mass of O_2 was calculated for each 1- to 2-m depth stratum. We adjusted the O_2 mass of the 0- to 1-m stratum for ice cover by subtracting the volume of ice present, assuming 100% O_2 expulsion from the ice, and estimating the O_2 mass in the remaining volume of water. Oxygen masses for all strata were summed to yield a total lake O_2 mass for each sample day. We estimated areal O_2 concentrations (grams per square metre) by dividing the total lake O_2 mass by the surface area of the water–ice interface.

Several lakes exhibited an O_2–time curve that was initially invariant for a period after freezing. To attempt to remove the possible input of O_2 via freeze-out (FO) and photosynthesis (PS) and only deal with decay rates, we consecutively removed initial O_2 concentration data and refit the nonlinear models to the remaining O_2 data (Fig. 2). At each step, we calculated the regression *F* value, and data were no longer removed once the highest *F* value was achieved. No data were removed if the complete set of O_2 data yielded the highest *F* value. If a time series of less than four O_2 concentrations were available, no data were removed.

Estimates of O_2 produced via FO and PS were calculated for seven lakes for the period over which data were removed. Estimates of O_2 expelled into the water column during freezing were calculated based on initial fall O_2 concentration and the ice depth on the day for which the last point was removed. Estimates of FO assume 100% expulsion of O_2 into the underlying water column upon freezing (Welch 1974) and that the O_2 concentration remained constant at the maximum fall O_2 concentration for the time over which FO was estimated. FO was calculated from freezing to the day the last point was removed (or as close to as possible), approximately 42–88 days after the lakes froze. This method likely yields slight overestimates of O_2 production via FO. We estimated total O_2 inputs by two different methods to obtain a maximum and minimum range of possible O_2 production. For method I, we calculated the difference in O_2 mass between the measured data and predicted (one-pool single exponential decay model projected back to the *y*-intercept) O_2 depletion curves (Fig. 1). Back-calculated O_2 concentrations were, with one exception, as high as four times the equilibrium concentrations. For method II, we assumed that O_2 production equaled O_2 losses over the initial phase, resulting in the initially flat curve. We assumed that O_2 was lost at the rate determined by the one-pool exponential decay model after point removal and that the O_2 concentration remained at the initial concentration for the duration of the phase. Oxygen inputs due to PS were estimated by subtracting the mass of O_2 produced via FO from the estimate of total O_2 inputs.

All analyses were performed with general linear models and nonlinear models procedures in SAS (v. 8.0). We fit each model to winter O_2 concentrations for each lake and evaluated the relative fits by visual examination of the residuals and summary statistics (e.g., *F* value). Linear O_2 depletion rates (eq. 1) were calculated for Manitoba and two Alberta lakes (Stephenson's Pond and Peterson's Meadow Pond) as per Barica and Mathias (1979) and Baird et al.

Fig. 2. Measured and predicted (one-pool) O_2 depletion curves for three typical Manitoba study lakes illustrating the effect of point removal. (*a*) Lake 255, 1974; (*b*) Lake 855, 1974; (*c*) Lake 675, 1974. Open circles, points removed; solid line, predicted curve (no points removed); broken line, predicted curve (points removed).



(1987) where rates were calculated for only the linear portion of the decay curve. Linear depletion rates calculated for all other lakes included all available data. For all lakes, the independent variable, time, began with day 0 at freezing. The model with the highest *F* value was deemed to have the best fit. To assess the correlation between lake and watershed characteristics and calculated O_2 depletion rates, we performed stepwise and backwards multiple regression. The lakes were split into groups with and without points removed to assess the robustness of our conclusions to point removal.

Results

Empirical curve fitting

The one-pool exponential decay model (eq. 2) relating areal O_2 concentration (grams per square metre) to time fit all lakes and years better than the linear model (judged by visual examination of the residuals) and yielded *F* values substantially higher than those of the linear model (eq. 1) (Table 2). The mean *F* value (±1 SD) for the one-pool exponential decay model (361.5 ± 588.9) was nearly eight times greater than that of the linear model (46.6 ± 66.0). It was not possible to fit the two-pool model to winter O_2 concentrations for all study lakes, but in those cases (22 of 41) where the model did fit, the *F* value was generally similar to or higher than that of the one-pool model. As the number of available O_2 data increased, the ability to fit the two-pool model increased, as evidenced by higher *F* values. In several of the cases where the model could not be fit, there was nonconvergence of the model, indicating that it was not possible to resolve the parameters. For other cases, there were simply too few data to yield parameter estimates.

The average parameter values (±1 SD) estimated by the two-pool model ($B + C = 2.0 \times 10^{36} \pm 9.5 \times 10^{36}$, $k_1 = 4.8 \times 10^6 \pm 2.2 \times 10^7$, $k_2 = 2.9 \times 10^5 \pm 1.4 \times 10^6$) were highly variable in comparison with one-pool model estimates ($A = 148.8 \pm 288.0$, $k = 0.035 \pm 0.076$). The distribution for each parameter, with the exception of *k* for the one-pool model, was not normal.

Point removal

Data from the early portion of the O_2–time curve were removed to attempt to correct for O_2 inputs between freezing and the establishment of permanent snow cover. Exclusion of one to four data (Fig. 2) increased *F* values substantially (mean *F* value (±1 SD) increased from 361 ± 589 to 814 ± 1822), although not all lakes benefited from point removal. In some cases, too few data were available, and point removal was not possible (e.g., Wisconsin LTER lakes). In other cases, point removal did not increase *F* values.

Freeze-out and photosynthesis

Estimates of FO and PS suggest that there were O_2 inputs for 40–88 days after freezing (Table 3). Inputs via FO were on average 5% of the total estimated inputs, and there was little interannual variability in the mass of O_2 inputs due to FO. Estimation of absolute O_2 input from PS by method I varied more than by method II, but PS was always 88–100% of total O_2 input regardless of the estimation method.

Correlation between *k* and lake and watershed features

Oxygen depletion rates for all lakes and years correlated most strongly with the sediment surface area to volume ratio (SSA:VOL) (Table 4). Stepwise and backwards regressions yielded the same final models for each total lake set and lakes with points removed. However, two different models were specified for lakes without points removed. Mean depth (z_{mean}) and surface area correlated with k for lakes with points removed. When morphometric variables were removed from the model, chlorophyll a (Chl a), fall O_2 concentration, and specific conductivity still failed to correlate with k. For lakes without points removed, Chl a and Secchi depth were the strongest correlates with k.

Lakes with points removed were smaller and shallower, with high surface area to volume ratio (SA:VOL) and SSA: VOL. These lakes also had higher specific conductivity and maximum Chl a (Table 5). The O_2 depletion rate for LTER lake Crystal Bog, 1982, was an order of a magnitude higher than k for all other lakes, poorly fit the single exponential model (F value = 0.06, p = 0.8296), and was removed from the lake set as an outlier. Crystal Bog is dystrophic, likely has high inputs of OM from its peat margins (T. Kratz, University of Wisconsin-Madison, Boulder Junction, WI 54512, U.S.A., personal communication), and is atypical for the lakes included in our analysis.

Discussion

Empirical curve fitting

Winter O_2 depletion in temperate zone lakes is best described by nonlinear models. Oxygen consumption was best modeled as a first-order nonlinear (single exponential decay) process that is most likely dominated by OM decay. The F values produced by the one- and two-pool models were comparable when both models fit the data, but two depletion rates were discernable in only some of the lakes. This does not mean that two or more pools of OM do not exist, but rather that it is not statistically possible to separate them with the data that we culled from the literature. The ability to fit the two-pool model increased as the number of available data increased, indicating that high resolution of the decay curve is necessary in order to estimate depletion rates of multiple pools of OM. Even when it was possible to fit the two-pool model, there was high variability in the estimated parameters. Fall O_2 concentrations and decay rates as estimated by the two-pool model covered a considerably broader range than reported in the literature (Barica and Mathias 1979; Babin and Prepas 1985; Prowse and Stephenson 1986). In an attempt to circumvent the inability of statistical software to estimate two rates simultaneously, we tried a curve-splitting technique (Riggs 1963) whereby one rate is estimated for a portion of the curve and then inserted into the entire model and held constant while the second rate is estimated. We were still not able to resolve the parameters for the unknown rate.

Point removal

The three alternative models that we fit do not capture the initial portion of the O_2–time curve. This is not surprising given that the models that we fit only address O_2 consumption and do not account for any O_2 inputs. For several study lakes, successive removal of one to four initial O_2 concentrations produced a much better fit for the nonlinear models as judged by F values and visual inspection of the residuals. For these lakes, inputs balance losses for 40 days on average. FO and PS should substantially influence O_2 dynamics under ice. That different lakes required a different number of points to be removed indicates that there is variability in the rate of O_2 inputs relative to O_2 depletion via consumptive processes and high variability between lakes in the time course over which these processes occur. Once inputs have ceased, O_2 consumption becomes the only process influencing O_2 dynamics, and the one-pool model best estimates the O_2 decay.

Freeze-out and photosynthesis

Dissolved O_2 has been observed to remain constant or to increase slightly during the first 15 days after freeze-up (Barica and Mathias 1979; Jackson and Lasenby 1982; Trimbee and Prepas 1988). Winter O_2 dynamics are a balance between processes that produce O_2, such as FO and PS, and those that consume O_2, such as decay of OM and chemical oxidation of reduced species. When water freezes, there is virtually 100% expulsion of gases from the ice into the underlying water (Welch 1974). As the ice thickens, underlying water is insulated from freezing temperatures. Ice thickening and FO are greatest for 30–40 days after initial freeze-up (Prowse and Stephenson 1986). Our estimates of PS and FO suggest that FO actually injects a very small amount of O_2 into the water relative to the total mass present at freezing and relative to inputs via PS in this set of lakes.

We calculated the primary O_2 inputs to be due to PS under the ice. Barica and Mathias (1979) indicated that the Manitoba lakes that we used are small, shallow lakes with no permanent surface inflow or outflow; therefore, inflow under ice via streams or groundwater is unlikely. Between freezing and persistent snow cover, substantial light is transmitted through the ice if it is relatively clear (Prowse and Stephenson 1986). Phytoplankton and nonsenescent macrophytes continue to photosynthesize and produce O_2. The duration and magnitude of O_2 production following freeze-up depend on how rapidly the ice thickens, how clear the ice is, and the presence–absence of snow. Water temperature remains between 0 and 4°C under ice, and temperature variation should therefore contribute little to variation in O_2 production. As the ice thickens, it increasingly attenuates light. The presence and depth of snow further decrease light transmittance. Prowse and Stephenson (1986) estimated that 12 cm of snow cover was sufficient to reduce the amount of light reaching underlying water to 1% of the incident light. Similarly, Welch and Kalff (1974) determined that 10–30 cm of snow was sufficient to reduce light transmission by 95–99%. Schindler and Nighswander (1970) found that phytoplankton were present under 0.5 m of ice with snow cover, but light levels were too low to facilitate detectable PS. Low temperatures also slow the rate of PS; however, most studies indicate that in the winter under ice, most algal PS is inhibited primarily by low light (Welch and Kalff 1974). Once snow cover is permanent, O_2 inputs via PS decline and O_2 dynamics become primarily a function of OM decay. Our results suggest that for the period over which O_2 data were removed, O_2 production equals consumption because O_2 concentrations do not change. This provides an estimate of how long inputs continue after freezing and averaged 40 days for the temperate zone lakes that we culled from the literature.

Climate and morphometry determine the extent to which O_2 production via FO and PS influences the entire lake O_2 balance. Inputs due to FO varied little because the processes underlying FO are the same from lake to lake. Lakes freeze

Table 2. Regression results for linear one- and two-pool models fit to winter areal O_2 concentrations ($g \cdot m^{-2}$) over time.

			Linear: $O_2 = A - k(t)$				One-pool: $O_2 = Ae^{-kt}$					
Lake set	Lake	Year	n	k	F	p	n	PR	A	k	F	p
MAN	19	1976	6	0.189	18.26	0.0079	5	1	54.56	0.013	388.30	0.0002
MAN	200	1976	4	0.279	47.89	0.0062	4	1	84.40	0.022	100.71	0.0098
MAN	255	1974	9	0.155	70.08	<0.0001	8	2	115.24	0.055	943.47	<0.0001
MAN	255	1975	11	0.135	54.33	<0.0001	11	1	70.36	0.038	284.94	<0.0001
MAN	255	1976	9	0.147	31.10	0.0005	8	2	1816.69	0.069	11469.5	<0.0001
MAN	318	1974	10	0.102	37.91	0.0002	11	0	17.85	0.012	86.33	<0.0001
MAN	318	1975	10	0.117	52.66	<0.0001	10	1	36.99	0.033	793.12	<0.0001
MAN	318	1976	3	0.120	4.35	0.1725	3	1	42.46	0.023	40.66	0.1102
MAN	587	1974	6	0.174	31.99	0.0024	5	2	54.66	0.017	622.79	0.0001
MAN	587	1975	9	0.208	216.37	<0.0001	8	2	96.60	0.029	206.65	<0.0001
MAN	587	1976	6	0.168	13.85	0.0137	5	2	108.22	0.022	323.39	0.0003
MAN	675	1974	10	0.246	105.70	<0.0001	7	4	682.04	0.044	257.41	<0.0001
MAN	675	1975	7	0.279	91.63	<0.0001	7	1	65.91	0.024	289.24	<0.0001
MAN	675	1976	4	0.328	31.44	0.0112	4	1	122.87	0.030	192.83	0.0052
MAN	721	1974	8	0.178	82.34	<0.0001	8	1	33.80	0.034	244.31	<0.0001
MAN	721	1975	8	0.142	41.20	0.0004	8	1	71.80	0.039	103.06	<0.0001
MAN	721	1976	5	0.182	42.21	0.0029	5	1	171.09	0.061	322.75	0.0003
MAN	882	1975	6	0.221	56.47	0.0007	6	1	151.98	0.054	419.95	<0.0001
MAN	882	1976	6	0.166	45.46	0.0011	6	1	68.62	0.035	288.28	<0.0001
MAN	885	1974	10	0.191	128.29	<0.0001	9	2	50.68	0.031	460.06	<0.0001
MAN	885	1975	7	0.178	82.92	<0.0001	7	1	68.83	0.040	171.09	<0.0001
MAN	885	1976	6	0.124	26.88	0.0035	6	1	74.92	0.047	744.71	<0.0001
MAN	Nora	1975	4	0.384	79.80	0.0030	4	0	95.04	0.014	136.24	0.0073
AB	STP	1984	9	–0.018	0.21	0.6602	8	0	17.11	0.067	496.40	<0.0001
AB	PMP	1984	11	–0.006	0.02	0.8965	9	0	24.93	0.048	425.07	<0.0001
AB	Les	1983	8	0.244	355.61	<0.0001	9	0	42.51	0.011	449.70	<0.0001
AB	Nak	1983	9	0.279	74.08	<0.0001	10	0	39.54	0.025	1738.4	<0.0001
LTER	BM	1981	3	0.086	0.09	0.7910	3	0	133.00	0.006	32.37	0.1233
LTER	BM	1982	3	0.057	0.16	0.7297	3	0	129.90	0.003	1902.0	0.0162
LTER	CB	1981	3	–0.019	0.73	0.4830	3	0	5.17	0.011	6.68	0.2638
LTER	CB	1982	4	–0.017	3.93	0.1417	3	0	69.04	0.500	0.06	0.8296
LTER	CR	1981	2	0.514	5.85	0.2496	3	0	171.80	–0.004	223.70	0.0472
LTER	CR	1982	3	–0.078	0.37	0.6064	3	0	137.10	0.001	795.30	0.0251
LTER	SP	1981	3	0.137	0.29	0.6428	3	0	166.80	0.005	469.74	0.0326
LTER	SP	1982	3	0.028	0.02	0.8903	3	0	143.70	0.003	749.27	0.0258
LTER	TB	1981	3	0.071	1.65	0.3279	3	0	51.84	0.018	55.69	0.0943
LTER	TB	1982	5	–0.004	0.00	0.9533	4	0	30.79	0.008	241.57	0.0041
LTER	TR	1981	3	0.578	1.18	0.3909	3	0	367.00	0.006	272.91	0.0428
LTER	TR	1982	3	–0.119	0.15	0.7344	3	0	322.10	0.004	df too small	
ONT	Coon	1975	12	0.166	82.10	<0.0001	11	1	50.28	0.013	2813.92	<0.0001
ELA	239	1968	10	0.147	13.37	0.0053	9	0	117.40	0.002	1341.32	<0.0001
ELA	240	1968	9	0.101	23.21	0.0013	9	0	72.54	0.002	2465.85	<0.0001

Note: Linear rates for Manitoba (MAN) and Alberta (AB: STP and PMP) lakes were calculated as per Barica and Mathias (1979) and Baird et al. (1987). increased. Sample size (n) and number of points removed (PR) are indicated for each study lake and sample year. NC indicates that the model failed to

at similar water temperatures and levels of O_2 saturation. Variability in the rate of FO would be due to ambient temperature, which is a function of weather in the initial days following freezing and determines how quickly the ice thickens. Most of the variability in the rate of O_2 produced is therefore due to PS. Oxygen production occurs at the water–ice interface and typically extends no more than 1–2 m below the ice due to light limitation (Schindler and Nighswander 1970). Oxygen consumption via respiration occurs throughout the entire water column. In shallow lakes with high SA:VOL, such as the lakes with points removed, inputs have a large impact on the total O_2 balance, likely because production at the water–ice interface influences a larger proportion of the complete water column than deep lakes with low SA:VOL. This pattern is evident by the initially flat curve after freezing. In large, deep lakes, O_2 inputs still occur, but they may be relatively small so as to have much less influence on the overall O_2 balance. We expect that shallow lakes should be influenced to a high degree by inputs following freezing but that with increasing depth, O_2 inputs should be

Two-pool: $O_2 = Be^{-k_1 t} + Ce^{-k_2 t}$							
n	PR	*B*	*C*	k_1	k_2	*F*	*p*
5	1			NC	NC		
5	0	-5.7×10^{33}	84	4.632	0.02	110.28	0.009
7	3	4.1×10^{2}	1.5×10^{9}	0.073	4072.80	1482.23	<0.0001
10	2	1.9×10^{2}	-4.5×10^{37}	0.054	2.03	422.75	<0.0001
8	2	-1.5×10^{13}	2.3×10^{4}	0.387	0.09	30813.7	<0.0001
10	1	22	0.91	0.017	–0.00	29.14	0.0005
9	2	31	-5.6×10^{3}	0.030	177.50	1267.8	<0.0001
4	0			NC	NC		
5	2			NC	NC		
8	2			NC	NC		
5	2	-6.7×10^{20}	2.3×10^{2}	0.656	0.03	319.63	0.0031
7	4	-7.0×10^{7}	6.8×10^{2}	1.1×10^{5}	0.04	257.41	<0.0001
6	2			NC	NC		
4	1	1.2×10^{2}	-1.7×10^{5}	0.030	136.80	192.83	0.0052
7	2	-1.0×10^{3}	1.1×10^{3}	0.027	0.03	243.45	<0.0001
8	1	1.6×10^{2}	14	0.067	0.02	39.58	0.0018
4	2			NC	NC		
4	3			NC	NC		
5	2			NC	NC		
8	3	69	-5.9×10^{9}	0.036	6.5×10^{6}	424.01	<0.0001
5	3	79	4.4×10^{27}	0.049	0.86	391.44	0.0025
7	0	76	-1.1×10^{29}	0.047	4.68	1521.4	<0.0001
4	0	2.9×10^{4}	95	6.429	0.01	136.24	0.0073
8	0	16	1.0	0.079	0.00	1848.1	<0.0001
9	0	25	2.9×10^{-3}	0.049	–0.04	185.55	<0.0001
9	0	6.6×10^{9}	43	1.9×10^{6}	0.01	449.70	<0.0001
8	2	36	1.2×10^{2}	0.024	0.13	1998.0	<0.0001
3	0			Too few data			
4	0			NC	NC		
3	0			NC	NC		
3	0			Too few data			
4	0			NC	NC		
4	0			NC	NC		
4	0			NC	NC		
4	0			NC	NC		
3	0			Too few data			
4	0			NC	NC		
3	0			Too few data			
3	0			Too few data			
11	1	8.5×10^{12}	50	1.0×10^{8}	0.01	2813.92	<0.0001
8	1	1.1×10^{2}	22	0.007	–0.01	538.70	<0.0001
6	1	77	-1.9×10^{11}	0.003	1.56	9179.99	<0.0001

Initial O_2 concentration points were removed when fitting the one- and two-pool models until *F* values no longer converge.

less noticeable, as exhibited by a steeper initial curve. After some critical depth, O_2 inputs will no longer have a noticeable impact and the O_2 balance should be primarily a function of OM decay from freeze to thaw. A rough estimate of this critical depth after which O_2 inputs likely will not be detected for temperate zone lakes, as determined by visual inspection of O_2 depletion curves, is a mean depth of 4–5 m.

The duration and magnitude of O_2 inputs after freezing clearly affect the O_2 mass balance and may be crucial to whether or not a lake goes anoxic. A longer input duration will extend the time over which O_2 inputs balance losses. Once inputs stop, the mass of O_2 in the lake and the rate of O_2 consumption set the time to anoxia. The time between freezing and persistent snow cover was, on average, longer for the shallow lakes, allowing O_2 inputs to occur for a longer time period. As a consequence, the duration of snow cover is shorter in these lakes, even though the period of ice duration is substantially longer than for deep lakes (~25 days). Shallow lakes are typically found on the prairies and in other areas of low relief and experience snow removal via

Table 3. Estimates of O_2 produced after freezing via PS and FO for select Manitoba lakes requiring point removal.

			Method I					Method II		
Lake	Years	FO ($g \cdot m^{-2}$)	P ($g \cdot m^{-2}$)	M ($g \cdot m^{-2}$)	P − M ($g \cdot m^{-2}$)	PS ($g \cdot m^{-2}$)	% O_2 input due to PS	Total inputs ($g \cdot m^{-2}$)	PS ($g \cdot m^{-2}$)	% O_2 input due to PS
255	1974–1975	1.4	182	50	132	131	99	47	45	97
	1975–1976	1.7	87	36	51	49	97	53	51	97
	1976–1977	2.1	559	58	501	499	100	118	116	98
318	1975–1976	1.6	42	29	12	11	87	31	30	95
	1976–1977	2.9	60	32	28	25	90	25	22	88
587	1974–1975	1.8	122	77	45	44	96	17	16	89
	1975–1976	1.6	125	68	57	55	97	66	65	98
	1976–1977	2.4	178	77	101	98	98	37	34	93
675	1974–1975	2.2	1172	137	1034	1032	100	61	59	96
	1975–1976	1.7	207	62	145	143	99	48	46	96
	1976–1977	3.3	118	68	50	46	93	73	70	95
721	1974–1975	2.4	53	39	13	11	82	30	27	92
	1975–1976	1.7	81	32	49	48	97	63	61	97
	1976–1977	2.8	81	29	53	50	95	77	74	96
882	1975–1976	0.5	114	35	79	78	99	93	92	99
	1976–1977	1.0	57	37	20	19	95	54	53	98
885	1974–1975	1.4	104	63	41	39	96	31	30	95
	1975–1976	1.6	84	36	48	46	97	59	57	97
	1976–1977	1.5	51	28	23	22	94	59	57	97

Note: In method I, the area between predicted (P) and measured (M) curves yields an estimate of the amount of total O_2 produced during the period between freezing and persistent snow cover. Subtracted from this is the total mass of O_2 produced via FO, yielding an estimate of O_2 produced by PS. Method II assumes that O_2 consumption equals O_2 production for the initial phase because inputs balance the respiration losses. Therefore, total inputs are a function of the decay rate (k), the initial O_2 concentration, and the duration of inputs. Total estimated inputs minus FO inputs yields an estimate of PS inputs.

Table 4. Stepwise and backwards multiple regression models for all lakes (total), lakes with points removed, and lakes with no points removed correlating k as estimated by the one-pool model with lake and watershed variables.

Lake set	Selection criteria	Model	df	F	R^2	p
Total	Stepwise	$k = 0.006 + 0.0005(\mathrm{SSA:VOL})$	34	45.58	0.58	<0.0001
	Backwards	$k = 0.006 + 0.0005(\mathrm{SSA:VOL})$	34	45.58	0.58	<0.0001
Points removed	Stepwise	$k = 0.069 + 0.01(z_{\mathrm{mean}}) - 8.16 \times 10^{-8}(\mathrm{SA})$	20	10.49	0.54	0.001
	Backwards	$k = 0.069 + 0.01(z_{\mathrm{mean}}) - 8.16 \times 10^{-8}(\mathrm{SA})$	20	10.49	0.54	0.001
No points removed	Stepwise	$k = 0.002 + 0.00041(\mathrm{Chl}\ a)$	12	26.28	0.70	0.0003
	Backwards	$k = 0.015 - 0.0015(\mathrm{Secchi}) - 0.00046(\mathrm{SSA:VOL})$	12	12.15	0.71	0.0021

Note: z_{mean} is mean depth (m), SA is surface area (m^2), SSA is sediment surface area (m^2), VOL is volume (m^3), and k is O_2 depletion rate ($g \cdot m^{-2} \cdot day^{-1}$).

higher wind action due to low relief and lack of trees in the surrounding watershed.

Empirical correlations

Decay rates in shallow lakes are most strongly correlated with lake morphometry. Shallow lakes have less water column through which suspended OM falls before it can be completely decomposed while in transit to the sediments. Consequently, the O_2 demand is great at the sediment–water interface (Cornett and Rigler 1987; Ellis and Stefan 1989). The nonsignificant correlation between Chl *a* and *k* after the removal of morphometry suggests that phytoplankton-derived carbon is not likely the dominant source of OM in all temperate lakes as historically believed (Charlton 1980; Mathias and Barica 1980). In macrophyte-dominated lakes, estimating the OM available for decomposition via Chl *a* likely leads to an underestimation of potential O_2 demand, and thus, Chl *a* by itself may be a poor predictor of O_2 depletion rates and anoxia in such lakes. Macrophytes can cover the entire lake bottom of shallow lakes, are a major source of OM once they begin to decompose in late summer and early fall (Chambers 1994), and should play an important role in O_2 depletion. Macrophyte biomass might therefore be a better correlate of O_2 decay rates in shallow lakes. Phytoplankton are most likely the main contributors of OM in deep lakes. This is consistent with the strong correlation between *k* and Chl *a* in lakes without points removed, which are also the largest and deepest lakes of the 23 included here.

Which model is most appropriate to describe winter O_2 depletion?

If the goals of modeling O_2 depletion are to gain an understanding of lake processes and to make predictions, it is important to incorporate O_2 dynamics from freeze to thaw. Important information concerning processes controlling O_2 dynamics exists in the initial and latter portions of the O_2–time curve. Describing the O_2–time curve directly after freezing conveys information on O_2 inputs. Describing the

Table 5. Mean (SD in parentheses) for lake and watershed characteristics for the total lake set, lakes with initial O_2 points removed, and lakes without initial O_2 points removed.

Variable	*n*	All lakes	*n*	Points removed	*n*	No points removed
k ($g \cdot m^{-2} \cdot day^{-1}$)	41	0.025 (0.019)	22	0.035 (0.015)	19	0.013 (0.017)
Fall $[O_2]$ ($g \cdot m^{-3}$)	41	11.51 (2.97)	22	11.30 (1.14)	19	11.75 (4.24)
z_{max} (m)	41	9.3 (9.5)	22	4.3 (3.3)	19	15.0 (11.1)
z_{mean} (m)	41	4.6 (4.1)	22	2.3 (0.7)	19	7.3 (4.8)
SA (m^2)	41	8.9×10^5 (1.9×10^6)	22	1.1×10^5 (1.2×10^5)	19	1.8×10^6 (2.5×10^6)
VOL (m^3)	41	9.6×10^6 (2.9×10^7)	22	2.5×10^5 (3.5×10^5)	19	2.0×10^7 (4.0×10^7)
SA:VOL (m^{-1})	41	0.383 (0.217)	22	0.509 (0.131)	19	0.237 (0.209)
SSA:VOL (m^{-1})	36	36.14 (28.96)	21	55.32 (14.14)	14	7.37 (20.01)
Secchi depth (m)	15	4.9 (2.9)	0	—	16	4.9 (2.9)
Specific conductance ($\mu mho \cdot cm^{-1}$)	25	1702 (996)	21	1855 (890)	4	895 (1275)
Summer Chl *a* ($mg \cdot m^{-3}$)	24	25.2 (22.1)	22	24.7 (22.4)	2	31.0 (25.0)
Maximum Chl *a* ($\mu g \cdot L^{-1}$)	38	51.6 (84.8)	21	78.4 (105.6)	17	18.5 (23.7)
Total phosphorus ($mg \cdot m^{-2}$)	4	89.0 (36.2)	0	—	4	89.0 (36.2)
Ice depth (cm)	41	64.0 (18.6)	22	69.9 (16.2)	19	57.1 (19.2)
Snow depth (cm)	39	23.7 (11.6)	22	25.0 (8.1)	17	22.0 (15.1)
Days between freeze and snow	28	35 (15)	22	40 (10)	6	16 (15)
Snow duration (days)	28	130 (15)	22	127 (13)	6	140 (19)
Ice duration (days)	39	168 (21)	22	178 (15)	17	154 (19)

depletion curve at O_2 concentrations <<1–3 $mg \cdot L^{-1}$ conveys information on the progress and extent of anoxia (Nürnberg 1995). Nonlinear estimates of k produce more accurate predictions than linear estimates of time to anoxia. Linear methods exclude the last portion of the O_2 concentration – time course. The one- and two-pool (where possible) exponential decay models capture O_2 dynamics in the latter tail, but none of the three models that we fit address the early portion of the O_2–time course for shallow lakes that experience noticeable O_2 inputs via FO and PS.

The most appropriate model to use ultimately depends on the types of questions addressed. The one-pool model should be used if estimates of fall O_2 concentrations and winter depletion rates are desired. Time to anoxia and questions regarding change in lake function and structure can all be addressed suitably with the one-pool model. The one-pool model sufficiently describes O_2 depletion rates while incorporating information in the end tail, which is probably the most critical for management purposes. Time to anoxia and magnitude and duration of anoxia can be easily predicted because parameter estimates are easily obtained and interpreted. Variability among estimated parameters can be attributed to environmental variation and not due to difficulty in fitting the model. The one-pool model does not discriminate between types of OM decay. If the questions at hand require the breakdown and characterization of O_2 demand of individual carbon pools (e.g., labile versus refractive), then multiple-pool models are required. For example, the two-pool model suggests that there are two distinct pools of OM that can be partitioned into relatively quickly and slowly decaying rates. Given the difficulty in fitting the two-pool model, it is necessary to have a finely resolved decay curve that derives from frequent (i.e., weekly) sampling.

Implications of anoxia

Oxygen dynamics vary greatly among lakes. Anoxia alters lake functions. Through negative feedbacks, changes in water quality can be associated with changes in rate processes and loss of trophic structure. Anaerobic respiration causes internal mobilization of phosphorus from sediments, increasing rates of phosphorus cycling (Nürnberg 1984). Eutrophication causes increased rates of O_2 depletion (Welch 1992) that in turn lead to higher probabilities of anoxia and fishkills.

Fishkills change lake trophic structure, which can affect productivity and water quality (Tonn and Magnuson 1982; Carpenter et al. 1985; DeAngelis and Cushman 1990). Loss of a top predator may cause shifts between clear, macrophyte-dominated and turbid, phytoplankton-dominated states in shallow lakes (Scheffer et al. 1993). In shallow lakes dominated by piscivorous fish, fishkills can result in a shift to a turbid state (Jeppesen et al. 1998). Phytoplankton blooms cause shading (Spence 1982) and can lead to loss of macrophyte beds and suitable fish and invertebrate habitat.

Predictions of O_2 depletion rates are important for maintenance and preservation of stocked and natural fisheries. Suitability of lakes for stocking and the feasibility of implementing lake aeration are often determined based on O_2 depletion rates. If accurate estimates of time to anoxia can be calculated, the suitability of lakes for stocking can be determined.

Acknowledgements

We thank Drs. J. Magnuson and B. Benson at the University of Wisconsin-Madison for providing data on Wisconsin lakes. We also thank M. Charlton, B. Nelsen, Dr. A. Paul, and two anonymous reviewers for improving this manuscript through their constructive reviews. This research was funded by a thesis research grant to M.E.M. and a Natural Sciences and Engineering Research Council of Canada operating grant to L.J.J.

References

Babin, J., and Prepas, E.E. 1985. Modelling winter oxygen depletion rates in ice-covered temperate zone lakes in Canada. Can. J. Fish. Aquat. Sci. **42**: 239–249.

Baird, D.J., Gates, T.E., and Davies, R.W. 1987. Oxygen conditions in two prairie pothole lakes during winter ice cover. Can. J. Fish. Aquat. Sci. **44**: 1092–1095.

Barica, J., and Mathias, J.A. 1979. Oxygen depletion and winterkill risk in small prairie lakes under extended ice cover. J. Fish. Res. Board Can. **36**: 980–986.

Brunskill, G.J., and Schindler, D.W. 1971. Geography and bathymetry of selected lake basins, Experimental Lakes Area, Northwestern Ontario. Can. J. Fish. Aquat. Sci. **28**: 139–155.

Burns, N.M. 1995. Using hypolimnetic dissolved oxygen depletion rates for monitoring lakes. N.Z. J. Mar. Freshwater Res. **29**: 1–11.

Carpenter, S.R., Kitchell, J.F., and Hodfson, J.R. 1985. Cascading trophic interactions and lake productivity. BioScience, **35**: 634–639.

Carpenter, S.R., Fisher, S.G., Grimm, N.B., and Kitchell, J.F. 1992. Global change and freshwater ecosystems. Annu. Rev. Ecol. Syst. **23**: 119–139.

Chambers, P.A. 1994. Submersed macrophytes in the Canadian prairies: dealing with home-grown problems. Lake Reservoir Manage. **10**: 5–8.

Charlton, M.N. 1980. Hypolimnion oxygen consumption in lakes: discussion of productivity and morphometry effects. Can. J. Fish. Aquat. Sci. **37**: 1531–1539.

Cornett, R.J. 1989. Predicting changes in hypolimnetic oxygen concentrations with phosphorus retention, temperature and morphometry. Limnol. Oceanogr. **34**: 1359–1366.

Cornett, R.J., and Rigler, F.H. 1987. Vertical transport of oxygen into the hypolimnion of lakes. Can. J. Fish. Aquat. Sci. **44**: 852–858.

Crosby, J.M., Bradford, M.E., Mitchell, P.A., Prepas, E.E., McIntyre, L.G, Buckland-Nicks, L., Hart, L., and Hanson, J.M. 1990. Atlas of Alberta lakes. University of Alberta Press. Edmonton, Alta.

DeAngelis, D.L., and Cushman, R.M. 1990. Potential applications of models in forecasting the effects of climate change on fisheries. Trans. Am. Fish. Soc. **119**: 224–239.

Ellis, C.R., and Stefan, H.G. 1989. Oxygen demand in ice covered lakes as it pertains to winter aeration. Water Resour. Bull. **25**: 1169–1176.

Gelda, R.K., and Auer, M.T. 1996. Development and testing of a dissolved oxygen model of a hypereutrophic lake. Lake Reservoir Manage. **12**: 165–179.

Greenbank, J.T. 1945. Limnological conditions in ice-covered lakes, especially related to winter-kill of fish. Ecol. Monogr. **15**: 344–392.

Jackson, M.B., and Lasenby, D.C. 1982. A method for predicting winter oxygen profiles in ice-covered Ontario lakes. Can. J. Fish. Aquat. Sci. **39**: 1267–1272.

Jeppesen, E., Søndergaard, M., Jensen, J.P., Kanstrup, E., and Petersen, B. 1998. Macrophytes and turbidity in brackish lakes with special emphasis on the role of top-down control. *In* The structuring role of submerged macrophytes in lakes. Ecological Studies. Vol. 131. *Edited by* E. Jeppesen, M. Søndergaard, M. Søndergaard, and K. Christoffersen. Springer-Verlag New York Inc., New York. pp. 369–377.

Lind, O.T. 1987. Spatial and temporal variation in hypolimnetic oxygen deficits of a multidepression lake. Limnol. Oceanogr. **32**: 740–744.

Mathias, J.A., and Barica, J. 1980. Factors controlling oxygen depletion in ice-covered lakes. Can. J. Fish. Aquat. Sci. **37**: 185–194.

McQueen, D.J., Post, J.R., and Mills, E.L. 1986. Trophic relationships in freshwater pelagic ecosystems. Can. J. Fish. Aquat. Sci. **43**: 1571–1581.

Nürnberg, G.K. 1984. The prediction of internal phosphorus loading in lakes with anoxic hypolimnion. Limnol. Oceanogr. **29**: 111–124.

Nürnberg, G.K. 1995. Quantifying anoxia in lakes. Limnol. Oceanogr. **40**: 1100–1111.

Prowse, T.D., and Stephenson, R.L. 1986. Relationship between winter lake cover, radiation receipts, and the oxygen deficit in temperate lakes. Atmos.–Ocean ATOCDA, **24**: 386–403.

Rahel, F.J. 1984. Factors structuring fish assemblages along a bog lake successional gradient. Ecology, **65**: 1276–1289.

Riggs, D.S. 1963. The mathematical approach to physiological problems. Williams & Wilkins Co., Baltimore, Md. pp. 146–161.

Scheffer, M., Hosper, H., Meijer, M.-L., Moss, B., and Jeppesen, E. 1993. Alternative equilibria in shallow lakes. Trends Ecol. Evol. **8**: 275–279.

Schindler, D.W. 1971. Light, temperature, and oxygen regimes of selected lakes in the Experimental Lakes Area, Northwestern Ontario. Can. J. Fish. Aquat. Sci. **28**: 157–169.

Schindler, D.W., and Nighswander, J.E. 1970. Nutrient supply and primary production in Clear Lake, eastern Ontario. J. Fish. Res. Board Can. **27**: 2009–2036.

Snodgrass, W.J., and Ng, P.S. 1985. Biochemical models for the hypolimnetic oxygen depletion models in lakes impacted by watershed discharges: 1. Alternative models. Arch. Hydrobiol. **1**(Suppl. 72): 81–109.

Spence, D.H.N. 1982. The zonation of plants in freshwater lakes. Adv. Ecol. Res. **12**: 37–125.

Tonn, W.M., and Magnuson, J.J. 1982. Patterns in the species composition and richness of fish assemblages in northern Wisconsin lakes. Ecology, **63**: 1149–1166.

Trimbee, A.M., and Prepas, E.E. 1988. Dependence of lake oxygen depletion rates on maximum oxygen storage in a partially meromictic lake in Alberta. Can. J. Fish. Aquat. Sci. **45**: 571–576.

Welch, E.B. 1992. Ecological effects of wastewater — applied limnology and pollutant effects. 2nd ed. Chapman and Hall, New York.

Welch, H.E. 1974. Metabolic rates of arctic lakes. Limnol. Oceanogr. **19**: 65–73.

Welch, H.W., and Kalff, J. 1974. Benthic photosynthesis and respiration in Char Lake. J. Fish. Res. Board Can. **31**: 609–620.

Differences in Cd accumulation among species of the lake-dwelling biomonitor *Chaoborus*[1]

Marie-Noële Croteau, Landis Hare, and André Tessier

Abstract: We measured substantial differences in Cd accumulation among four species of the phantom midge *Chaoborus* that were exposed in the laboratory to the same Cd concentration in naturally contaminated prey. The two large-bodied species accumulated more Cd than did the two small-bodied species, in spite of the fact that all species ingested prey at the same rate. To determine why this was the case, we fitted our experimental data to a bioaccumulation model that allowed us to compare the species with respect to their rate constants for growth and Cd efflux, their Cd assimilation efficiency, and their Cd concentrations at steady state. Differences among species were explained mainly by the fact that the small-bodied species assimilated a much lower proportion of the Cd that they ingested with prey (≈6%) than did the large-bodied species (45 and 58%). A comparison between Cd concentrations measured in *Chaoborus* species in the field and predictions from the model suggests that differences in Cd concentrations among coexisting *Chaoborus* species in nature are explained by differences both in the rate at which they assimilate Cd and in their feeding habits.

Résumé : Nous avons mesuré des différences substantielles dans l'accumulation du Cd chez quatre espèces de *Chaoborus* (Mouche-fantôme) qui avaient été exposées en laboratoire à des quantités similaires de Cd contenues dans des proies naturellement contaminées. Au terme de cette expérience, les deux espèces de grande taille avaient accumulé plus de Cd que les deux espèces de petite taille et ce, malgré un taux d'ingestion des proies similaire chez les quatre espèces. Afin de déterminer les causes de cette accumulation différentielle du Cd entre espèces, nous avons appliqué à nos données expérimentales un modèle de bioaccumulation théorique. Ce modèle nous a permis de comparer les espèces en regard de leurs constantes de taux de croissance et de perte de Cd, de leur efficacité d'assimilation du Cd et de leurs concentrations de Cd à l'état stationnaire. Les différences mesurées dans l'accumulation du Cd entre les espèces furent expliquées principalement par le fait que les espèces de petite taille assimilaient de leurs proies une proportion de Cd beaucoup plus faible (≈6%) que les espèces de grande taille (45 et 58%). La comparaison des prédictions de notre modèle aux concentrations de Cd mesurées chez des espèces de *Chaoborus* récoltées en milieu naturel suggère que les différences observées en milieu naturel dans les concentrations de Cd entre les espèces coexistantes sont attribuables au fait que les espèces diffèrent entre elles tant dans leur efficacité d'assimilation du Cd que dans leur régime alimentaire.

Introduction

Interest in trace metal accumulation by aquatic organisms stems in part from their potential use as biomonitors (Phillips and Rainbow 1993). In both marine and freshwater systems, bivalve mollusks are widely used as sentinel animals for monitoring contaminant levels (Elder and Collins 1991; Rainbow and Phillips 1993). However, most bivalve species are absent from waters of pH below 6 (Pennak 1989) and their metal concentrations could be subject to the confounding influence of a reproductive cycle (Langston and Spence 1995). Moreover, the use of many freshwater bivalves for monitoring purposes is limited by the dependence of their larval stage on the presence of suitable host fish (Clarke 1981) and their sensitivity to trace metals (Couillard et al. 1993). The phantom midge *Chaoborus* has been proposed as an alternative metal biomonitor in lakes (Hare and Tessier 1996). Larvae of this insect occur over a wide range of chemical conditions (Hare and Tessier 1996, 1998; Croteau et al. 1998), are widely distributed (Borkent 1981), and can be abundant (Yan et al. 1985). Furthermore, *Chaoborus* larvae are able to accumulate and tolerate high concentrations of trace metals (Hare and Tessier 1996, 1998; Croteau et al. 1998) and their metal content does not change during a reproductive cycle (Hare and Campbell 1992).

The use of an animal as a biomonitor depends on the establishment of a reliable relationship between the animal's metal concentration and the concentration of the metal in its surroundings. Hare and Tessier (1996) tested such a relationship for larvae of *Chaoborus punctipennis* in a large number of lakes. They demonstrated that Cd concentrations in this insect are best described by the free-ion activity model, provided that competition for biological uptake sites between H ions and free Cd ions are explicitly taken into account. Further studies showed that several species of *Chaoborus* could be grouped together for biomonitoring purposes, obviating the need for microscopic examination of individuals

Received November 28, 2000. Accepted June 6, 2001. Published on the NRC Research Press Web site at http://cjfas.nrc.ca on August 9, 2001.
J16106

M.-N. Croteau, L. Hare,[2] and A. Tessier. Institut National de la Recherche Scientifique-Eau (INRS-Eau), Université du Québec, C.P. 7500, Sainte-Foy, QC G1V 4C7, Canada.

[1] Contribution from the Metals in the Environment Research Network (MITE-RN).

[2] Corresponding author (e-mail: landis@inrs-eau.uquebec.ca).

Can. J. Fish. Aquat. Sci. **58**: 1737–1746 (2001) DOI: 10.1139/cjfas-58-9-1737

(Croteau et al. 1998; Hare and Tessier 1998). However, the performance of this generic model was reduced compared with that for a single species (*C. punctipennis*) because Cd concentrations differed among coexisting *Chaoborus* species (Croteau et al. 1998; Hare and Tessier 1998). Specific differences in Cd concentrations have also been reported among sympatric congeners of a marine copepod (*Calanus* spp.; Ritterhoff and Zauke 1997).

Cadmium bioaccumulation can be influenced by physical (e.g., temperature), chemical (e.g., trace metal speciation), ecological (e.g., diet, life history), and physiological factors (e.g., Cd assimilation efficiency, Cd loss rate). Few studies have compared the processes that govern metal uptake and loss among related animal species. In the present study, we used a novel experimental approach to determine if larvae of four species of the phantom midge *Chaoborus* accumulate Cd differently when environmental and ecological factors are held constant. That is, we wished to determine if there are physiological differences among species that could influence their accumulation of this metal. We exposed the various *Chaoborus* species to naturally contaminated prey of the same type and Cd content in a controlled environment. Then, using a bioaccumulation model, we estimated and compared key food-related parameters, such as Cd assimilation efficiency and Cd loss rate, among species. Lastly, we compared model predictions with Cd concentrations measured in field-collected *Chaoborus*.

Methods

Our measurements of Cd concentrations and dynamics were performed on four *Chaoborus* species that are widespread in North America (Borkent 1981): *C. albatus*, *C. americanus*, *C. flavicans*, and *C. punctipennis*.

Collection of field samples

For our Cd uptake experiment, larvae of four species of *Chaoborus* were collected in early September 1998 from three low-Cd lakes, two of which were located near Québec City and one (Hélène) in the Rouyn-Noranda region upwind from a metal smelter (Table 1). For our Cd loss experiment, larvae of four *Chaoborus* species were collected in mid-September 1998 from three high-Cd lakes located in the Rouyn-Noranda area (Table 1). Larvae for the experiments were sampled after sunset by hauling a 250-µm plankton net horizontally in the water column. Water samples were collected using in situ diffusion samplers (peepers) similar to those described by Carignan et al. (1985). These Plexiglas samplers comprise eight compartments of 4 mL each that were filled with ultrapure water (Milli-Q system water, >18 Mohm·cm^{-1}) and separated from the lake water by a 0.2-µm polysulfone membrane (Gelman HT-200 membrane). After preparation, each sampler was sealed in a clean plastic bag prior to its placement in a lake. Two diffusion samplers were suspended 1 m above the bottom in the epilimnion of each lake. After a 3-day equilibration period, the diffusion samplers were retrieved and water was collected immediately for the measurement of Cd.

Samples (4 mL) for Cd analyses were removed from compartments in each diffusion sampler by piercing the membrane with a pipette fitted with an acid-cleaned tip. These samples were injected into preacidified (53 µL of 1.35 N Anachemia nitric acid) high-density polyethylene (HDPE) bottles (4 mL capacity). On installation and retrieval dates, pH was measured with a portable pH meter (Hanna instruments, microprocessor model HI9024/HI9025) in water samples collected at the depth of the diffusion samplers using a Van Dorn bottle.

For comparison of Cd concentrations among *Chaoborus* species, larvae were obtained from 17 Canadian Shield lakes containing two to four species of the genus (Table 2). Larvae were collected either with an Ekman grab in sediment during the day (1992 and 1993 samples) or with a 250-µm-mesh-aperture plankton net during the night (1996 and 1997 samples). In the laboratory, *Chaoborus* larvae were sorted according to species (Saether 1972) and final (fourth) instar larvae were differentiated by head capsule length, as given in Larow and Marzolf (1970) (*C. punctipennis*), Fedorenko and Swift (1972) (*C. americanus*), and Parma (1971) (*C. flavicans*). Given the similar size and morphology of *C. albatus* and *C. punctipennis* larvae (they belong to the same subgenus; Borkent 1979), we assumed that head capsule lengths were the same for these two species. Larvae were held in filtered lake water for 24 h prior to metal analysis to allow them to defecate their gut contents. Five to 20 individuals of a given species were pooled, to minimise potential individual variations in Cd, and placed on a piece of preweighed acid-washed Teflon sheeting and then frozen until analysis.

Internal Cd distribution

To measure the internal distribution of Cd in larvae, *Chaoborus* from several lakes (i.e., Lake Marlon for *C. punctipennis* and *C. albatus* larvae, Lake Caron for *C. flavicans* larvae, and Lake Turcotte for *C. americanus* larvae (Table 1)) were dissected under a microscope into gut and remaining body parts; organs from 10–15 larvae of a given species were pooled to make a single sample, and five such samples were prepared for each *Chaoborus* species. Larvae were not given time to defecate their gut contents prior to dissection because a preliminary experiment conducted at 5°C showed that there was no significant difference in Cd concentrations between larvae that were allowed to eliminate their gut contents for 24 h and those that were not (Fig. 1).

Cadmium uptake experiment

Twenty-five final-instar larvae of each *Chaoborus* species collected from low-Cd lakes (i.e., Lake Hélène for *C. punctipennis* and *C. albatus*, Lake Laflamme for *C. flavicans*, and Lake Bertrand for *C. americanus* (Table 1)) were placed individually into 30-mL HDPE bottles filled with filtered (64 µm) water from Cd-contaminated Lake Marlon (Table 1). From this same lake, we collected the widespread calanoid copepod *Skistodiaptomus oregonensis* using a 64 µm mesh size plankton net to offer as prey to *Chaoborus* larvae. We verified daily the efficacy of our prey sorting by identifying prey to be offered to *Chaoborus* in three randomly selected vials (nominally holding 25 prey each). All individuals examined were calanoid copepods, of which 90% were *S. oregonensis* and 10% were the similar-sized *Epischura lacustris*. Prey were mainly adults (36%) and copepodite V (45%), with the remainder being either copepodite IV or copepodite III. Adults and copepodite V should be eaten in similar numbers, given their similar body width (0.25 ± 0.02 (SD) mm), and readily, given their small width compared with the mouth diameter of *Chaoborus* (0.45 mm for fourth instars of our smallest species, *C. punctipennis*; Moore 1988).

Each *Chaoborus* larva was offered 25 fresh prey daily, which is in excess of its needs (larvae of a given species consumed an average of 12–44% of prey offered). *Chaoborus* larvae were held in the dark at 5°C throughout the Cd uptake experiment. Both filtered lake water and prey were renewed every 24 h by transferring each larva to a new bottle filled with freshly collected filtered lake water and copepods. Formalin (5% final solution) was added to the previous 24-h-exposure bottles to preserve uneaten copepods for later counting under a microscope. On the basis of these samples, we calculated daily ingestion rates for each *Chaoborus* larva.

Table 1. Location of the lakes from which we collected final-instar *Chaoborus* larvae for the uptake and loss experiments as well as their mean (±SD) Cd concentrations.

Lake (region)	Location	pH	[Cd] (nM)	Species	Cd concentration (μg·g^{-1})
Cd uptake experiment					
Bertrand (Québec City)	46°58′N, 72°01′W	5.01	0.36	*C. americanus*	1.44±0.17
Laflamme (Québec City)	47°19′N, 71°07′W	6.71	0.09	*C. flavicans*	0.46±0.02
Hélène (Rouyn-Noranda)	48°13′N, 79°10′W	7.76	0.06	*C. punctipennis*	0.18±0.04
				C. albatus	0.22±0.05
Cd loss experiment					
Turcotte (Rouyn-Noranda)	48°18′N, 79°04′W	5.26	8.06	*C. americanus*	14.4±1.0
Caron (Rouyn-Noranda)	47°56′N, 78°58′W	7.18	0.82	*C. flavicans*	6.0±1.0
Marlon (Rouyn-Noranda)	48°16′N, 79°04′W	7.48	1.08	*C. punctipennis*	4.9±1.9
				C. albatus	3.2±1.4

Note: Also given are lake water pH and total dissolved Cd concentration.

Table 2. Location of the study lakes and year of sampling as well as the Cd concentrations in final-instar *Chaoborus* larvae collected in the spring (May–June).

			Cd concentration (μg·g^{-1})			
Region and lake	Location	Year	*C. punctipennis*	*C. albatus*	*C. flavicans*	*C. americanus*
Sudbury, Ont.						
Crooked	46°25′N, 81°02′W	1997	3.2±0.3 *a*	3.8	4.0±0.6 *b*	4.0±0.2 *b*
Crowley	46°23′N, 80°59′W	1997	10.9±0.3 *a*	np	8.6±0.4 *b*	np
Forest	46°23′N, 81°00′W	1997	8.3±0.3 *a*	np	4.9±0.2 *b*	np
Hazen*	46°25′N, 80°59′W	1996	1.1±0.1 *a*	np	1.6±0.1 *b*	2.6±0.4 *c*
Jonny	46°26′N, 81°02′W	1997	11.9±0.7 *a*	np	np	13.6±0.8 *b*
Pine*	46°22′N, 81°02′W	1996	3.9±1.2 *a*	3.4±0.1 *a*	np	np
Tilton	46°22′N, 81°04′W	1997	7.6±0.8 *a*	np	6.6±0.5 *a*	np
Rouyn-Noranda, Qué.						
Bousquet	48°14′N, 78°34′W	1997	2.0±0.1 *a*	np	3.0±0.4 *b*	np
Caron	47°56′N, 78°58′W	1996	4.5±0.3 *a*	np	7.4±0.5 *b*	np
Desperiers*	48°11′N, 79°09′W	1996	1.1±0.2 *a*	np	2.8±0.3 *b*	np
Duprat	48°20′N, 79°08′W	1997	1.4±0.1 *a*	np	1.3±0.1 *a*	np
Hélène†	48°13′N, 79°10′W	1993	0.24±0.03 *a*	0.31±0.04 *a*	np	np
Joannès†	48°11′N, 78°41′W	1992	2.6±0.3 *a*	np	3.4±0.5 *a*	np
Marlon	48°16′N, 79°04′W	1997	5.6±0.5 *a*	4.9±0.5 *a*	np	np
Surimeau*	48°08′N, 79°19′W	1996	2.7±0.2 *a*	np	1.3±0.1 *b*	np
Turcotte*	48°18′N, 79°04′W	1996	3.1±0.1 *a*	np	np	9.9±0.7 *b*
Vaudray	48°07′N, 78°42′W	1997	2.9±0.2 *a*	np	4.5±0.2 *b*	np

Note: Cd concentration values are means ± 95% confidence interval of usually several (n = 1–8) samples of pooled final-instar larvae. np, not present. Means of sympatric species followed by a different letter are significantly different (t test, $p < 0.05$).

*Data from Croteau et al. (1998).

†Data from Hare and Tessier (1998).

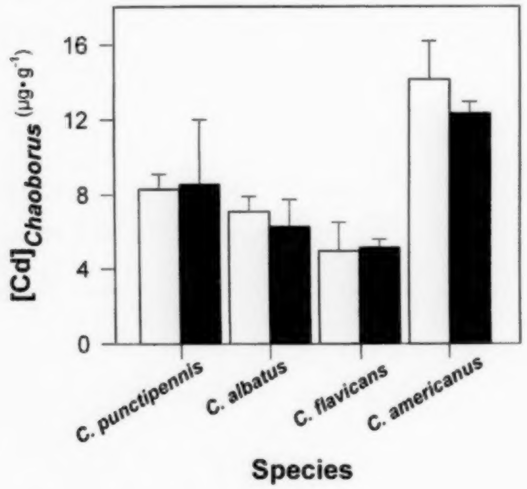
On each day, Cd concentrations were measured in three replicate samples of 100–150 prey. Pooled prey samples were held on preweighed acid-washed Teflon sheeting in microcentrifuge tubes and frozen at –4°C until analysis. At the beginning of the experiment and after 2, 4, 7, 10, and 14 days of Cd exposure, five undepurated larvae (see Fig. 1) of each *Chaoborus* species were placed individually on a piece of preweighed acid-washed Teflon sheeting and frozen.

Cadmium loss experiment

Fifty final-instar larvae of each *Chaoborus* species collected from high-Cd lakes (i.e., Lake Marlon for *C. punctipennis* and *C. albatus*, Lake Caron for *C. flavicans*, and Lake Turcotte for *C. americanus* (Table 1)) were placed individually into 30-mL HDPE bottles filled with filtered (64 μm) water from low-Cd Lake Hélène (Table 1). These *Chaoborus* larvae were fed ad libidum bulk zooplankton (mainly copepods) collected from low-Cd Lake Hélène using a 64-μm-mesh-size plankton net. *Chaoborus* larvae were held in the dark at 5°C throughout the Cd loss experiment. Both filtered lake water and prey were renewed every 24 h by transferring each larva to a new bottle filled with freshly collected filtered lake water and bulk plankton. On each day, three replicate samples of bulk zooplankton (≈1–2 mg wet weight) were placed on a piece of preweighed acid-washed Teflon sheeting and frozen until analysis. At times 0, 6, 12, 18, 24, and 36 h and 2, 3, 5, 7, and 10 days, five undepurated larvae (see Fig. 1) of each *Chaoborus* species were placed individually on pieces of preweighed acid-washed Teflon sheeting and frozen until analysis.

In the lakes from which we collected our *C. americanus* for both Cd uptake and loss experiments, we found two body sizes of *C. americanus* larvae, both of which had the same head capsule length (corresponding to that of the fourth instar). This suggests that there are two coexisting generations of larvae of different ages (≈1 and 2 years of age). For our experiments, we chose the smaller

Fig. 1. Mean Cd concentrations (+95% confidence interval) in larvae of the four *Chaoborus* species both soon after their capture for use in our Cd loss experiment (gut contents present, open bars) and following a 24-h depuration period (gut contents absent, solid bars).



of the two larval sizes so as to minimise the risk of emergence during our experiment and so that their age would be close to those of the other three species (<1 year).

Analyses

To minimise inadvertent trace metal contamination, labware, water-sampling materials, vials, and Teflon sheeting were soaked in 15% nitric acid and rinsed in ultrapure water prior to use.

Total dissolved Cd concentrations were measured by flameless atomic absorption spectrophotometry (Perkin-Elmer model SIMAA 6000). Certified reference water samples (riverine water reference material, NRCC) were also analysed for Cd during each analytical run and measured trace metal concentrations were within the certified range.

Previously frozen *Chaoborus* larvae and zooplankton samples were freeze-dried (FTS Systems^{TM}), weighed (Mettler ME30 electronic microbalance), and digested in concentrated nitric acid (Aristar grade). For samples of *Chaoborus* collected before 1998, digestions were carried out either in thick-walled, screw-cap Teflon vials in an autoclave at 120°C for 3 h or in Teflon bombs in a microwave oven. Cooled digested samples were diluted to volume with ultrapure water. Samples of both *Chaoborus* and copepods collected in 1998 were digested at room temperature in 4-mL HDPE vials with concentrated nitric acid (100 μL·mg dry weight sample^{-1}) for 7 days. Hydrogen peroxide (40 μL·mg dry weight sample^{-1}) was added 24 h prior to final dilution with ultrapure water (760 μL·mg dry weight sample^{-1}). Samples of similar weight of a certified reference material (lobster hepatopancreas, TORT-1, NRCC) were submitted to the same digestion procedures during each analytical run. Cadmium concentrations measured in TORT-1 were within the certified range and recoveries of Cd in spiked samples were within ±10% of the amount added. Cadmium concentrations in animals were analysed by flameless atomic absorption spectrophotometry (Varian Spectra AA-30). The software SYSTAT 10 was used for all statistical analyses. We used *t* tests to compare Cd concentrations between the gut and remaining organs as well as to compare estimates for each model term among *Chaoborus* species. Estimates for each model term in the Cd uptake and Cd loss experiments were made by nonlinear regression.

Results and discussion

Internal Cd distribution

A comparison of the distribution of Cd between the gut and remaining organs confirmed that the four *Chaoborus* species that we studied are similar in having the bulk of their Cd localised in gut tissues (*t* test, $p < 0.001$) (Fig. 2). This internal distribution pattern suggests that all of the species take up the bulk of their Cd from prey. This suggestion is borne out for one of our study species, *C. punctipennis*, by laboratory and field studies demonstrating that larvae of this species accumulate almost all of their Cd from food (Munger and Hare 1997; Munger et al. 1999). Because the Cd present in *Chaoborus* larvae likely originates from their food, then differences in Cd concentrations among sympatric species could be explained by the type and quantity of prey that each species consumes. In addition, factors unrelated to food type and ingestion rate, such as Cd assimilation efficiency, Cd elimination rate, and animal growth rate, could vary among species and thereby explain the differences in Cd concentrations among *Chaoborus* species that are reported to occur in nature (Croteau et al. 1998; Hare and Tessier 1998).

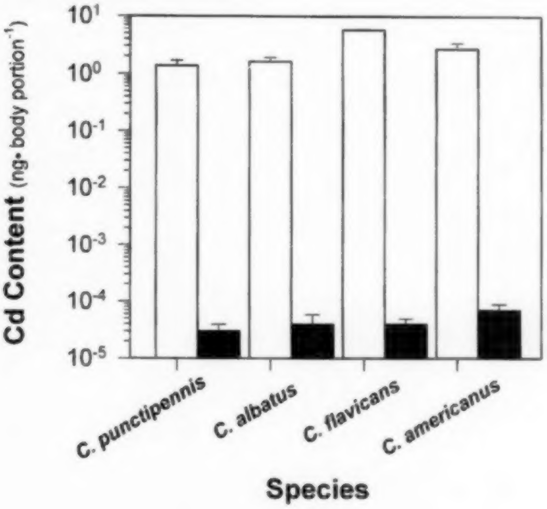
Modelling Cd bioaccumulation

We tested the hypothesis that if all species consume the same prey type at the same rate, their Cd concentrations will increase to the same extent. We did this by feeding four *Chaoborus* species the calanoid copepod *S. oregonensis* collected from the same Cd-rich lake. We used naturally contaminated prey so that Cd accumulation by the predator would be representative of that that occurs in the field; the availability of Cd from food is reported to depend on the exposure history of the food particles (Wallace and Lopez 1996). Despite the fact that the *Chaoborus* species took up their Cd from the same prey type, they differed significantly (*t* test, $p < 0.001$) in the rates at which they accumulated Cd, i.e., 9 ng Cd·g^{-1}·day^{-1} for *C. albatus*, 16 ng Cd·g^{-1}·day^{-1} for *C. punctipennis*, 74 ng Cd·g^{-1}·day^{-1} for *C. americanus*, and 109 ng Cd·g^{-1}·day^{-1} for *C. flavicans* (SD < 1 for all species). To explain this difference among species, we examined variables other than prey type and prey Cd concentration that are known to influence metal uptake: ingestion rate (IR), Cd assimilation efficiency (AE), Cd efflux rate, and *Chaoborus* growth rate (Wang and Fisher 1996; Munger et al. 1999; Munger and Hare 2000).

Mean ingestion rates (grams dry weight copepods consumed per gram dry weight predator per day) (Table 3) did not differ significantly among *Chaoborus* species during our Cd uptake experiment (*t* test, $p > 0.05$) (with the exception of a slightly lower value for *C. albatus* (*t* test, $p = 0.004$)). This similarity in ingestion rates among predator species suggests that prey ingestion rate cannot explain the differences in Cd accumulation rates that we observed among species in our Cd uptake experiment. To assess the influence of assimilation efficiencies, Cd efflux rates, and larval growth rates on the differences that we measured among *Chaoborus* species, we fit the following bioaccumulation model to our experimental data.

If we treat larvae as a single compartment and assume that their Cd uptake from water is negligible, as discussed above,

Fig. 2. Mean Cd contents (+ 95% confidence interval) in the gut (open bars) and remaining body parts (solid bars) of larvae of the four *Chaoborus* species.



we can express the rate of change in larval Cd concentrations as the difference between Cd entering and leaving larvae provided that we take into account Cd dilution due to animal growth (Thomann 1981):

$$(1)\quad \frac{\mathrm{d}[\mathrm{Cd}]_{Chaoborus}}{\mathrm{d}t} = \underbrace{(\mathrm{AE}\times\mathrm{IR}\times[\mathrm{Cd}]_{\mathrm{Food}})}_{\text{Cd uptake from food}} - \underbrace{(k_{\mathrm{e}}[\mathrm{Cd}]_{Chaoborus})}_{\text{Cd loss by efflux}} - \underbrace{(k_{\mathrm{g}}[\mathrm{Cd}]_{Chaoborus})}_{\text{Cd dilution by growth}}$$

where AE is the Cd assimilation efficiency (grams Cd retained per gram Cd ingested), $[\mathrm{Cd}]_{\mathrm{Food}}$ and $[\mathrm{Cd}]_{Chaoborus}$ (micrograms Cd retained per gram dry weight) are the Cd concentrations in prey and in *Chaoborus* larvae, respectively, and k_e and k_g (per day) are the rate constants for Cd efflux and animal growth, respectively.

Furthermore, we assumed that larval growth can be represented by the exponential function (Spacie and Hamelink 1985)

$$(2)\quad W = W_0\mathrm{e}^{k_g t}$$

where W_0 is the initial weight (milligrams dry weight per larva) and t is time (days). We then used eq. 2 and the integrated form of eq. 1 to estimate for each *Chaoborus* species the rate constants k_e and k_g as well as the Cd assimilation efficiency, as described below.

First, we used eq. 2 and our measurements of changes in larval mass during the Cd uptake experiment (Fig. 3*a*) to estimate, by nonlinear regression, values of W_0 and the growth rate constant k_g. Values of k_g were highest for the large-bodied species but low or negligible for the small-bodied species (Table 3). Low k_g values are not surprising given the low temperature (5°C) at which we conducted our experiments (Davies and Tribe 1969). Under similar feeding conditions but at higher temperatures, k_g values are substantially greater for these *Chaoborus* species (M.-N. Croteau, unpublished data). Our k_g values are in the range of those found for *C. punctipennis* (–0.001 to 0.020·day^{-1}) by Munger et al. (2000) and for a variety of soil arthropods (0.003–0.027·day^{-1}) by Crommentuijn et al. (1994). The fact that growth rate constants were higher for the large-bodied species suggests that this parameter cannot explain the higher increases in Cd concentrations of these species in our experiments; greater larval growth lowers Cd concentrations by dilution of the Cd in the added body tissues.

To estimate values of the Cd loss rate constant k_e, it was first necessary to estimate, by nonlinear regression, values of the growth rate constant for the loss experiment (k_g^*) (Table 3) using eq. 2 and our measurements of changes in larval mass during the Cd loss experiment (Fig. 3*b*). We then assumed that Cd uptake was negligible during our Cd loss experiment. This assumption is reasonable because we fed high-Cd *Chaoborus* larvae low-Cd prey; Cd concentrations in prey offered during the Cd loss experiment were 10 times lower than in those offered during the Cd uptake experiment (Table 3). This assumption allowed us to simplify eq. 1, which in its integrated form becomes

$$(3)\quad [\mathrm{Cd}]_{Chaoborus} = [\mathrm{Cd}]^{0*}_{Chaoborus}\mathrm{e}^{-(k_g^*+k_e)t}$$

Where $[\mathrm{Cd}]^{0*}_{Chaoborus}$ is the Cd concentration in larvae (micrograms Cd per gram dry weight) at the beginning of the Cd loss experiment. Using our measurements of Cd concentrations in *Chaoborus* during the Cd loss experiment (Fig. 4) and the k_g^* values from Table 3, we estimated the loss rate constant k_e by nonlinear regression (eq. 3). The Cd loss rate constants for our four *Chaoborus* species (0.003–0.037·day^{-1}) (Table 3) are in the range of those reported for a variety of invertebrates, i.e., 0.003–0.018·day^{-1} for *C. punctipennis* (Munger et al. 1999), 0.012·day^{-1} for mussels (Roditi and Fisher 1999), and 0.017–0.048·day^{-1} for soil arthropods (Crommentuijn et al. 1994). Differences in the loss rate constant among species are reported to depend on the physiology of the species involved (Crommentuijn et al. 1994). The fact that *C. americanus* larvae had the highest Cd loss rate constant does not explain why they accumulated more Cd than did the small-bodied species in the Cd uptake experiment.

Using the values of k_g and k_e from Table 3, the ingestion rates calculated daily for each *Chaoborus* larva of each species, as well as the mean daily Cd concentration measured in food offered during the uptake experiment, we estimated Cd assimilation efficiencies for the four species (Table 3) by fitting our data points (Fig. 5) to the integrated form of eq. 1:

$$(4)\quad [\mathrm{Cd}]_{Chaoborus} = \frac{\mathrm{AE}\times\mathrm{IR}\times[\mathrm{Cd}]_{\mathrm{Food}}}{k_g+k_e} \times\left(1-\mathrm{e}^{-(k_g+k_e)t}\right) + [\mathrm{Cd}]^{0}_{Chaoborus}\mathrm{e}^{-(k_g+k_e)t}$$

For the overall mean Cd concentration in prey offered to *Chaoborus* ($[\mathrm{Cd}]_{\mathrm{Food}}$), we used a value of 10.6 ± 0.3 (SD) μg·g^{-1} as given in Table 3. We note that this Cd concentration in prey is much higher than the highest Cd concentration that we measured in *Chaoborus* at the end of our uptake experiment, i.e., 1.8 μg·g^{-1} for *C. americanus*. This apparent "biodilution" of Cd in our experimental food chain

Table 3. Values of Cd accumulation parameters measured (±95% confidence interval) or estimated (±SE) from our experiments with the various *Chaoborus* species.

Parameter	Abbreviation or symbol	*C. punctipennis*	*C. albatus*	*C. flavicans*	*C. americanus*
Cd uptake experiment					
Initial *Chaoborus* weight (mg·larva^{-1}) (±95% CI)	W_0	0.23±0.04 *a* (n = 5)	0.24±0.02 *a* (n = 5)	0.66±0.15 *b* (n = 5)	0.44±0.07 *c* (n = 5)
Initial *Chaoborus* Cd concentration (µg·g^{-1}) (±95% CI)	$[\mathrm{Cd}]^{0}_{Chaoborus}$	0.18±0.04 *a* (n = 5)	0.22±0.05 *a* (n = 5)	0.46±0.02 *b* (n = 5)	1.44±0.15 *c* (n = 5)
Mean prey Cd concentration (µg·g^{-1}) (±95% CI)	$[\mathrm{Cd}]_{\mathrm{Food}}$	10.6±0.3 *a* (n = 49)	10.6±0.3 *a* (n = 49)	10.6±0.3 *a* (n = 49)	10.6±0.3 *a* (n = 49)
Mean ingestion rate (g prey·g predator^{-1}·day^{-1}) (±95% CI)	IR	0.052±0.009 *a* (n = 25)	0.038±0.005 *b* (n = 25)	0.057±0.008 *a* (n = 25)	0.046±0.007 *ab* (n = 25)
Growth rate constant (day^{-1}) (±SE)	k_g	0.0134±0.0082 *ab* (n = 25)	–0.0012±0.0092 *b* (n = 25)	0.0232±0.0058 *a* (n = 25)	0.0253±0.0046 *a* (n = 25)
Cd assimilation efficiency (%) (±SE)	AE	5.7±1 *a* (n = 25)	6.9±1 *a* (n = 25)	45±4 *b* (n = 25)	58±4 *c* (n = 25)
Cd loss experiment					
Initial *Chaoborus* weight (mg·larva^{-1}) (±95% CI)	W_0^*	0.22±0.04 *a* (n = 5)	0.29±0.04 *a* (n = 5)	0.61±0.18 *b* (n = 5)	0.24±0.03 *a* (n = 5)
Initial *Chaoborus* Cd concentration (µg·g^{-1}) (±95% CI)	$[\mathrm{Cd}]^{0*}_{Chaoborus}$	8.29±0.81 *a* (n = 5)	7.07±0.83 *a* (n = 5)	4.95±1.55 *b* (n = 5)	14.1±2.05 *c* (n = 5)
Final *Chaoborus* Cd concentration (µg·g^{-1}) (±95% CI)	$[\mathrm{Cd}]^{10*}_{Chaoborus}$	8.78±3.29 *ac* (n = 5)	4.63±1.67 *ab* (n = 5)	4.92±0.38 *b* (n = 5)	8.90±1.03 *c* (n = 5)
Mean prey Cd concentration (µg·g^{-1}) (±95% CI)	$[\mathrm{Cd}]^{*}_{\mathrm{Food}}$	1.1±0.1 *a* (n = 36)	1.1±0.1 *a* (n = 36)	1.1±0.1 *a* (n = 36)	1.1±0.1 *a* (n = 36)
Growth rate constant (day^{-1}) (±SE)	k_g^*	0.015±0.0118 *a* (n = 55)	–0.0051±0.0129 *a* (n = 54)	–0.0091±0.0112 *a* (n = 55)	–0.0019±0.0091 *a* (n = 55)
Loss rate constant (day^{-1}) (±SE)	k_e	0.0029±0.0164 *a* (n = 55)	0.0253±0.0172 *a* (n = 54)	0.0038±0.0090 *a* (n = 55)	0.0368±0.0085 *a* (n = 55)
Cd exchange rates (ng Cd·g^{-1}·day^{-1})					
Cd uptake rate	UR	$t_{0,10}$ = 3.2	$t_{0,10}$ = 2.6	$t_{0,10}$ = 28	$t_{0,10}$ = 29
Cd loss rate	LR	$t_{0,10}$ = 154, 163	$t_{0,10}$ = 143, 94	$t_{0,10}$ = –26	$t_{0,10}$ = 493, 344
Net change in Cd	ΔCd	–160 to –151	–140 to –91	54	–464 to –315

Note: Abbreviations or symbols marked with an asterisk refer to the Cd loss experiment. Values followed by a different letter are significantly different (t test, $p < 0.05$). Also given are Cd exchange rates during our Cd loss experiment on the basis of parameter estimates at $t = 0$ and $t = 10$.

Fig. 3. Temporal changes in the dry weight (±SD) of (*a*) low-Cd *Chaoborus* species larvae that were exposed for 14 days to water and copepods (ad libidum) from a Cd-rich lake and (*b*) high-Cd *Chaoborus* species larvae that were exposed for 10 days to water and bulk zooplankton (ad libidum) from a low-Cd lake. Experimental data are represented by symbols, whereas lines represent model curves obtained with eq. 2 and the parameters W_0, W_0^*, k_g, and k_g^* given in Table 3. Triangles and circles indicate values for the small-bodied species (open for *C. punctipennis* and solid for *C. albatus*) and the large-bodied species (open for *C. americanus* and solid for *C. flavicans*), respectively.

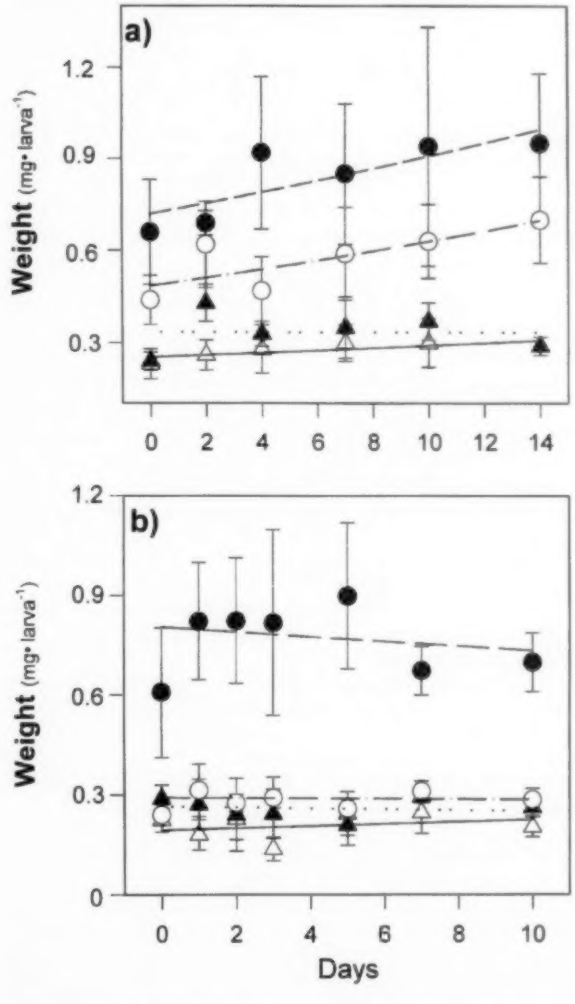
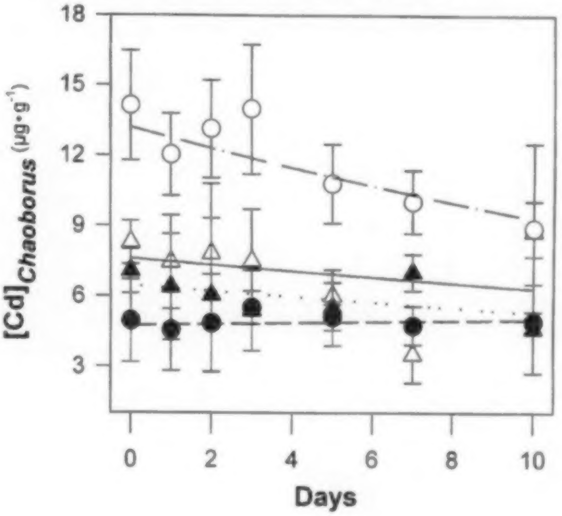


Fig. 4. Temporal changes in the Cd concentration (±SD) of high-Cd *Chaoborus* species larvae that were exposed for 10 days to water and bulk zooplankton (ad libidum) from a low-Cd lake. Experimental data are represented by symbols, whereas lines represent model curves obtained with eq. 3 and the parameters k_e, k_g^*, and $[\mathrm{Cd}]^{0*}_{Chaoborus}$ given in Table 3. Triangles and circles indicate values for the small-bodied species (open for *C. punctipennis* and solid for *C. albatus*) and the large-bodied species (open for *C. americanus* and solid for *C. flavicans*), respectively.

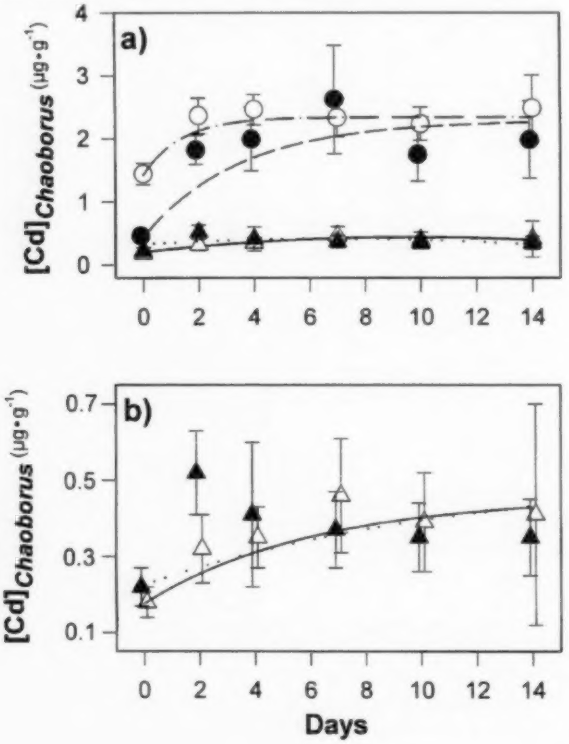


is reported to be common in nature (Hare 1992; C.Y. Chen et al. 2000).

Cadmium assimilation efficiencies differed substantially among species (Table 3), which suggests that it is mainly this variable that explains the species differences in Cd accumulation during our uptake experiment. Assimilation efficiencies were lowest for the small-bodied species *C. albatus* and *C. punctipennis* (6–7%) (Table 3); the value for the latter species (≈6%) is within the range reported for this taxon by Munger et al. (1999) in a field experiment (2–18%). The similarity of the Cd assimilation efficiency values for *C. albatus* and *C. punctipennis* is consistent with their similarity in body size, morphology, and migratory behaviour. Larvae of *C. flavicans*, although the largest in body size, had a somewhat lower assimilation efficiency (45%) than *C. americanus* (58%) (Table 3), the second largest of the four species. If species are ranked according to their gain in mass during our experiment, there is a statistically significant ($r^2 = 0.91$, $p = 0.045$) relationship with their Cd assimilation efficiency (Fig. 6). A similar correlation between the assimilation of C and that of Cd by larvae has been reported for a bivalve by Wang and Fisher (1996).

The explanation for species differences in Cd assimilation efficiencies likely resides in differences among the taxa in the manner in which they consume, digest, and assimilate prey. Species differences in Cd assimilation efficiency cannot be attributed to the degree to which they ingest prey either whole or in pieces (sloppy feeding) because (*i*) we usually found prey whole, albeit crushed, in the guts of all *Chaoborus* species, (*ii*) larval mouth parts are similar among species and do not appear to be designed to tear or dismember prey (Saether 1972), and (*iii*) *Chaoborus* are reported to be gape-limited predators, which suggests that they eat prey whole (Hare and Carter 1987). Following ingestion, prey are crushed in the pharynx, which is facilitated in some *Chaoborus* species by a pair of large teeth on the pharyngeal sphincter. Differences in teeth structure among species are reported to be related to the hardness of their prey (Hare and Carter 1987). All of the species that we studied possess a similar pair of simple spines on the pharyngeal sphincter

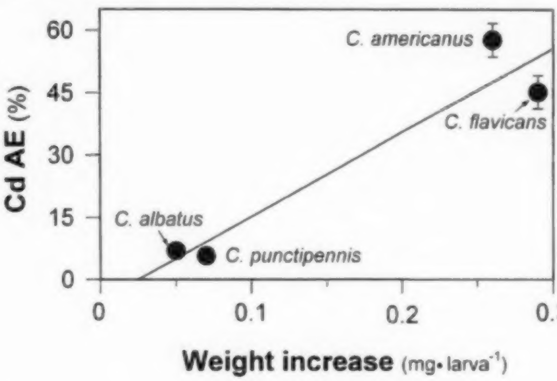
Fig. 5. Temporal changes in the Cd concentrations of *Chaoborus* species larvae from a Cd-poor lake that were exposed for 14 days to water and copepods (ad libidum) from a Cd-rich lake. In both panels, lines represent model curves (obtained with eq. 4 and the parameters assimilation efficiency, ingestion rate, W_0, k_g, k_e, and $[\mathrm{Cd}]^0_{Chaoborus}$ given in Table 3) and symbols (±SD) represent our experimental data. The data for *C. punctipennis* and *C. albatus* larvae are repeated in Fig. 5*b* with the scale on the vertical axis expanded. Triangles and circles indicate values for the small-bodied species (open for *C. punctipennis* and solid for *C. albatus*) and the large-bodied species (open for *C. americanus* and solid for *C. flavicans*), respectively.



(similar to those illustrated for *Chaoborus ceratopogones* by Hare and Carter 1987), which suggests that differences in Cd assimilation efficiency among our *Chaoborus* species cannot be explained by this feature. After solid prey parts have been egested via the mouth, remaining liquids pass down the digestive tract where differences in residence time (Decho and Luoma 1991), enzyme efficiencies (Mayer et al. 1996; Z. Chen et al. 2000), and both the number and the nature of Cd uptake sites in the gut (Hare 1992) could lead to differences in C and Cd assimilation efficiencies. Because information on these subjects is lacking for *Chaoborus*, we are unable to speculate further about the cause of the large differences in Cd assimilation efficiency among our study species.

We assumed that Cd uptake by *Chaoborus* larvae during our Cd loss experiment was negligible. We can test this assumption by using eq. 1 and values found in both our Cd uptake experiment (assimilation efficiency, ingestion rate, $[\mathrm{Cd}]_{\mathrm{Food}}$, k_g (Table 3)) and our Cd loss experiment (k_e, k_g^*, $[\mathrm{Cd}]^{0-10*}_{Chaoborus}$(Table 3)) to estimate specific Cd uptake and loss rates during our Cd loss experiment. Excluding *C. flavicans* (which had a negative Cd loss rate), Cd uptake rates (calculated at both $t = 0$ and $t = 10$) were lower than Cd loss rates by a factor of from 12 to 55 times (Table 3), confirming that Cd uptake was negligible compared with Cd loss during our loss experiment. The negative Cd loss rate for *C. flavicans* is due to the combination of a very low k_e value and weight gain by this species during the Cd loss experiment (Fig. 3*b*). Because the Cd uptake rates given in Table 3 suggest that all *Chaoborus* species accumulated some Cd during our Cd loss experiment, Cd loss rates for all species are likely underestimates.

Fig. 6. Cadmium assimilation efficiency (±SE) (also see Table 3) of *Chaoborus* species larvae as a function of their increase in dry weight during a 14-day exposure to both Cd-contaminated food and water ($r^2 = 0.91$, $p = 0.045$).



Differences in Cd concentrations among *Chaoborus* species in the field

When Cd concentrations in *Chaoborus* larvae reach a steady state ($[\mathrm{Cd}_{Chaoborus}]_{ss}$), i.e., when $d[\mathrm{Cd}]_{Chaoborus}/dt$ equals zero, eq. 1 becomes

$$(5) \qquad [\mathrm{Cd}_{Chaoborus}]_{ss} = \frac{\mathrm{AE} \times \mathrm{IR} \times [\mathrm{Cd}]_{\mathrm{Food}}}{k_e + k_g}.$$

Using the values of assimilation efficiency, ingestion rate, $[\mathrm{Cd}]_{\mathrm{Food}}$, and k_g from the Cd uptake experiment as well as those of k_e from the Cd loss experiment (Table 3), we estimated steady-state values of 10.1, 4.5, 1.9, and 1.1 µg Cd·g^{-1} for *C. flavicans*, *C. americanus*, *C. punctipennis*, and *C. albatus*, respectively. If we assume that in nature, coexisting species feed on the same prey type at the same rate, as in our experiments, we would expect to observe the following patterns among *Chaoborus* species in the field. Firstly, Cd concentrations in the two small-bodied species (*C. punctipennis* and *C. albatus*) should be similar, which was the case in the four lakes in which they coexisted (Table 2). This similarity in Cd accumulation is likely explained in part by the similar morphology of these species, which are placed apart from the large-bodied species in the subgenus *Sayomyia* (Borkent 1979). These small-bodied species could be treated as a unit for Cd monitoring purposes. Secondly, our laboratory experiments would lead us to expect that Cd concentrations in *C. americanus* larvae would exceed those in the two small-bodied species, which was the case in the four lakes in which these species coexisted (Table 2). Because of these differences, grouping the small- and large-bodied species together for biomonitoring purposes would likely reduce the accuracy of predictions, as we observed in our earlier studies (Croteau et al. 1998; Hare and Tessier 1998). Lastly, we would expect that Cd concentrations in *C. flavicans* larvae should exceed those of the three other species. This prediction was borne out in only four of our 12 study lakes (Table 2). Because of this departure from our expectations, it is likely that our assumption of similar Cd concentrations in the diets of *C. flavicans* and the other species is not tenable. Major differences in diet have been reported between sympatric populations of *C. flavicans* and *C. punctipennis* (Sardella and Carter 1983), but comparative dietary data on other coexisting species is lacking. Other biological differences among species, such as their migratory behaviour, could also influence their accumulation of Cd in nature. Among our study species, larvae of *C. americanus* spend all of their time in the warm epilimnion during summer. In contrast, the other species spend the day in the colder waters or sediment of the hypolimnion (to avoid fish predation) and migrate into warmer surface waters only at night to feed on zooplankton. Differences in the temperatures to which larvae of the various *Chaoborus* species are exposed could alter their metabolic rate and their assimilation of Cd and C (Giguère and Dill 1980; Giguère 1981).

Overall, our experimental results suggest that even with the same food source, Cd accumulation can differ substantially among *Chaoborus* species due in the main to differences in the efficiency with which they assimilate Cd from their food.

Acknowledgements

Funding was provided by the U.S. Environmental Protection Agency, the Metals in the Environment Research Network (MITE-RN), the Ontario Power Generating Corporation, the Mining Association of Canada, Human Resources Development Canada, the Natural Sciences and Engineering Research Council of Canada, and the Québec Fonds pour la Formation de Chercheurs et l'Aide à la Recherche. Technical assistance by M.G. Bordeleau, P. Fournier, P. Marcoux, R. Savard, S. St-Pierre, and R. Rodrigue is acknowledged.

References

Borkent, A. 1979. Systematics and bionomics of the species of the subgenus *Schadonophasma* Dyar and Shannon (*Chaoborus*, Chaoboridae, Diptera). Quaest. Entomol. **15**: 122–255.

Borkent, A. 1981. The distribution and habitat preferences of the Chaoboridae (Culicomorpha: Diptera) of the Holarctic region. Can. J. Zool. **59**: 122–133.

Carignan, R., Rapin, R., and Tessier, A. 1985. Sediment pore-water sampling for metal analysis: a comparison of techniques. Geochim. Cosmochim. Acta, **49**: 2493–2497.

Chen, C.Y., Stemberger, R.S., Klaune, F., Blum, J.D., Pickhardt, P.C., and Folt, C.L. 2000. Accumulation of heavy metals in food web components across a gradient of lakes. Limnol. Oceanogr. **45**: 1525–1536.

Chen, Z., Mayer, L.M., Quétel, C., Donard, O.F.X., Self, R.F.L., Jumars, P.A., and Weston, D.P. 2000. High concentrations of complexed metals in the guts of deposit feeders. Limnol. Oceanogr. **45**: 1358–1367.

Clarke, A.H. 1981. The freshwater molluscs of Canada. National Museum of Natural Sciences, Ottawa, Ont.

Couillard, Y., Campbell, P.G.C., and Tessier, A. 1993. Response of metallothionein concentrations in a freshwater bivalve (*Anodonta grandis*) along an environmental cadmium gradient. Limnol. Oceanogr. **38**: 299–313.

Crommentuijn, T., Doodeman, C.J.A.M., Doornekamp, A., Van Der Pol, J.J.C., Bedaux, J.J.M., and Van Gestel, C.A.M. 1994. Lethal body concentrations and accumulation patterns determine time-dependent toxicity of cadmium in soil arthropods. Environ. Toxicol. Chem. **13**: 1781–1789.

Croteau, M.-N., Hare, L., and Tessier, A. 1998. Refining and testing a trace metal biomonitor (*Chaoborus*) in highly acidic lakes. Environ. Sci. Technol. **32**: 1348–1353.

Davies, P.S., and Tribe, M.A. 1969. Temperature dependence of metabolic rate in animals. Nature (Lond.), **224**: 723–724.

Decho, A.W., and Luoma, S.N. 1991. Time-courses in the retention of food material in the bivalves *Potamocorbula amurensis* and *Macoma balthica*: significance to the absorption of carbon and chromium. Mar. Ecol. Prog. Ser. **78**: 303–314.

Elder, J.F., and Collins, J.J. 1991. Freshwater molluscs as indicators of bioavailability and toxicity of metals in surface-water systems. Rev. Environ. Contam. Toxicol. **122**: 37–79.

Fedorenko, A.Y., and Swift, M.C. 1972. Comparative biology of *Chaoborus americanus* and *Chaoborus trivittatus* in Eunice lake, British Columbia. Limnol. Oceanogr. **17**: 721–730.

Giguère, L.A. 1981. Food assimilation efficiency as a function of temperature and meal size in larvae of *Chaoborus trivittatus* (Diptera: Chaoboridae). J. Anim. Ecol. **50**: 103–109.

Giguère, L.A., and Dill, L.M. 1980. Seasonal patterns of vertical migration: a model for *Chaoborus trivittatus*. *In* Evolution and ecology of zooplankton community. *Edited by* W.C. Kerfoot. University Press of New England, Hanover, N.H. pp. 122–128.

Hare, L. 1992. Aquatic insects and trace metals: bioavailability, bioaccumulation, and toxicity. Crit. Rev. Toxicol. **22**: 327–369.

Hare, L., and Campbell, P.G.C. 1992. Temporal variations of trace metals in aquatic insects. Freshwater Biol. **27**: 13–27.

Hare, L., and Carter, J.C.H. 1987. Zooplankton populations and the diets of three *Chaoborus* species (Diptera, Chaoboridae) in a tropical lake. Freshwater Biol. **17**: 275–290.

Hare, L., and Tessier, A. 1996. Predicting animal cadmium concentrations in lakes. Nature (Lond.), **380**: 430–432.

Hare, L., and Tessier, A. 1998. The aquatic insect *Chaoborus* as a biomonitor of trace metals in lakes. Limnol. Oceanogr. **43**: 1850–1859.

Langston, W.J., and Spence, S.K. 1995. Biological factors involved in metal concentrations observed in aquatic organisms. *In* Trace metal speciation and bioavailability in aquatic systems. *Edited by* A. Tessier and D. Turner. Wiley, New York. pp. 407–478.

Larow, E.J., and Marzolf, G.R. 1970. Behavioral differences between 3rd and 4th instars of *Chaoborus punctipennis* Say. Am. Midl. Nat. **84**: 428–436.

Mayer, L.M., Chen, Z., Findlay, R.H., Fang, J., Sampson, S., Self, R.F.L., Jumars, P.A., Quetel, C., and Donard, O.F.X. 1996. Bioavailability of sedimentary contaminants subject to deposit-feeder digestion. Environ. Sci. Technol. **30**: 2641–2645.

Moore, M. V. 1988. Differential use of food resources by the instars of *Chaoborus punctipennis*. Freshwater Biol. **19**: 249–268.

Munger, C., and Hare, L. 1997. Relative importance of water and food as cadmium sources to an aquatic insect (*Chaoborus* *punctipennis*): implications for predicting Cd bioaccumulation in the field. Environ. Sci. Technol. **31**: 891–895.

Munger, C., and Hare, L. 2000. Influence of ingestion rate and food types on cadmium accumulation by the aquatic insect *Chaoborus*. Can. J. Fish. Aquat. Sci. **57**: 327–332.

Munger, C., Hare, L., and Tessier, A. 1999. Cadmium sources and exchange rates for *Chaoborus* larvae in the field. Limnol. Oceanogr. **44**: 1763–1771.

Parma, S. 1971. The morphology of the larval instars of *Chaoborus flavicans* (Meigen, 1818) (Diptera, Chaoboridae). Zool. Mus. Univ. Amsterdam, **18**: 173–181.

Pennak, R. 1989. Freshwater invertebrates of the United States. 3rd ed. Wiley, New York.

Phillips, D.J.H., and Rainbow, P.S. 1993. Biomonitoring of trace aquatic contaminants. Elsevier, London, U.K.

Rainbow, P.S., and Phillips, D.J.H. 1993. Cosmopolitan biomonitors of trace metals. Mar. Pollut. Bull. **26**: 593–601.

Ritterhoff, J., and Zauke, G.-P. 1997. Trace metals in field samples of zooplankton from the Fram Strait and the Greenland Sea. Sci. Total Environ. **199**: 255–270.

Roditi, H.A., and Fisher, N.S. 1999. Rates and routes of trace element uptake in zebra mussels. Limnol. Oceanogr. **44**: 1730–1749.

Saether, O.A. 1972. Chaoboridae. *In* Das Zooplankton der Binnengewässer. *Edited by* H.J. Elster and W. Ohle. E. Schweizerbart'sche, Stuttgart, Germany. pp. 257–304.

Sardella, L., and Carter, J.C.H. 1983. Factors contributing to coexistence of *Chaoborus flavicans* and *C. punctipennis* (Diptera, Chaoboridae) in a small meromictic lake. Hydrobiologia, **107**: 155–164.

Spacie, A., and Hamelink, J.L. 1985. Bioaccumulation. *In* Fundamentals of aquatic toxicology. *Edited by* G.M. Rand and S.R. Petrocelli. Hemisphere Publishing, New York. pp. 495–525.

Thomann, R.V. 1981. Equilibrium model of fate of microcontaminants in diverse aquatic food chains. Can. J. Fish. Aquat. Sci. **38**: 280–296.

Wallace, W.G., and Lopez, G.R. 1996. Relationship between subcellular cadmium distribution in prey and cadmium trophic transfer to a predator. Estuaries, **19**: 923–930.

Wang, W.-X., and Fisher, N.S. 1996. Assimilation of trace elements and carbon by the mussel *Mytilus edulis*: effects of food composition. Limnol. Oceanogr. **41**: 197–207.

Yan, N.D., Nero, R.W., Keller, W., and Lasenby, D.C. 1985. Are *Chaoborus* larvae more abundant in acidified than in non-acidified lakes in central Canada? Holarct. Ecol. **8**: 93–99.

Testing the functional redundancy of *Limnodrilus* and *Tubifex* (Oligochaeta, Tubificidae) in hyporheic sediments: an experimental study in microcosms

Florian Mermillod-Blondin, Magali Gérino, Valérie Degrange, Robert Lensi, Jean-Luc Chassé, Michael Rard, and Michel Creuzé des Châtelliers

Abstract: Most tubificid worms are classified in the same functional group by their similar bioturbation and feeding activities in fine sediments of lakes. The objective of this study was to test the functional redundancy of two genera of tubificids (*Limnodrilus* and *Tubifex*) at two densities in coarse sediments using slow filtration columns. We measured the effects of the worms on particle redistribution, organic matter processing, nutrient fluxes, and microbial characteristics. The results showed that *Limnodrilus* and *Tubifex* created the same stimulation of aerobic and anaerobic microbial activities in the sediment. However, 50 *Tubifex* had a greater effect than 50 *Limnodrilus* on microbial processes. Furthermore, at a density of 100 oligochaetes per column, only *Tubifex* significantly increased particle redistribution and oxygen consumption in the first centimetre of the sediment. In contrast, *Limnodrilus* more often modified microbial activity in the deeper layers of the columns. The functional redundancy of the two genera of tubificid worms was validated by most measured processes. However, the variability within functional groups cannot be neglected because each genus had its functional peculiarity in the ecosystem.

Résumé : En raison de leur mode de déplacement et d'alimentation dans les sédiments fins des lacs, la plupart des oligochètes tubificidés sont classés dans le même groupe fonctionnel. L'objectif de cette étude est de tester la redondance fonctionnelle entre deux genres de tubificidés (*Limnodrilus* et *Tubifex*) pour deux densités en organismes dans des sédiments grossiers en utilisant des colonnes infiltrantes. L'effet des vers est mesuré sur la redistribution des particules, la décomposition de la matière organique, les flux de nutriments et plusieurs paramètres microbiens. Les résultats indiquent que *Limnodrilus* et *Tubifex* produisent une stimulation des activités microbiennes aérobie et anaérobie dans le sédiment. Cependant, 50 *Tubifex* ont un effet plus important sur l'activité microbienne que 50 *Limnodrilus*. A une densité de 100 individus par colonne, seul *Tubifex* augmente significativement la redistribution des particules et la consommation en oxygène dans les premiers cm de sédiment. Au contraire, *Limnodrilus* semble être plus actif que *Tubifex* en profondeur. La redondance des deux genres de vers est validée par la plupart des processus mesurés. Cependant, la variabilité à l'intérieur des groupes fonctionnels ne peut être négligée car chaque genre a sa spécificité dans le fonctionnement de l'écosystème.

Introduction

In recent years, several investigators tried to link species diversity with ecosystem processes (Lawton 1994; Palmer et al. 1997). Studies dealing with the functional aspect of diversity have described the role of species in ecosystems by using the functional group concept. A functional group is a set of species having a similar role in ecosystem processes (Chapin et al. 1992).

In fine sediments of lakes (mud and fine sand), tubificid worms such as *Tubifex tubifex* and *Limnodrilus hoffmeisteri* have been classified in the functional group of conveyor-belt deposit feeders (McCall and Tevesz 1982). The activities of these worms in fine sediments (feeding and bioturbation)

Received October 23, 2000. Accepted June 8, 2001. Published on the NRC Research Press Web site at http://cjfas.nrc.ca on August 9, 2001.
J16031

F. Mermillod-Blondin[1] and M. Creuzé des Châtelliers. UMR-CNRS 5023, Ecologie des Hydrosystèmes Fluviaux, HydroBiologie et Ecologie Souterraines, Université Lyon I, 43 Bd du 11 Novembre 1918, 69622 Villeurbanne, France.
M. Gérino and M. Rard. UMR-CNRS 5576, Centre d'Ecologie des Systèmes Aquatiques Continentaux, 29 rue Jeanne Marvig, 31055 Toulouse, France.
V. Degrange and R. Lensi. UMR-CNRS 5557, Ecologie Microbienne, Université Lyon I, 43 Bd du 11 Novembre 1918, 69622 Villeurbanne, France.
J.-L. Chassé. UMR-CNRS 5558, Biométrie et Biologie Evolutive, Université Lyon I, 43 Bd du 11 Novembre 1918, 69622 Villeurbanne, France.

[1] Corresponding author (e-mail: mermillo@cismsun.univ-lyon1.fr).

DOI: 10.1139/cjfas-58-9-1747

play a significant role in the processing of organic matter and nutrient cycling in both aerobic and anaerobic conditions (Torreiter et al. 1994; van de Bund et al. 1994). However, in hyporheic habitats characterized by coarse sediments (gravel and sand) and convection flow of water into the sediment, the functional classification of worms has not been established yet because the effect of oligochaetes on system function remains poorly known (Boulton 2000). In a previous study performed in slow filtration columns filled with coarse sediments (gravel and sand), we showed a large effect of *Limnodrilus* tubificid worms on aerobic and anaerobic microbial activities (Mermillod-Blondin et al. 2000*a*).

The objective of the present study was to determine if the classification of tubificid worms established for lake sediments was also valid in hyporheic habitats. According to the redundancy model (Walker 1992), if two taxa of tubificids belong to the same functional group, they should create similar effects on ecosystem processes with a possible difference in effect intensity (François et al. 1999) or in effect location. In the present study, the redundancy within the tubificid group was investigated in hyporheic sediments using representatives of two genera (*Limnodrilus* and *Tubifex*) that are widely distributed and commonly co-occur in freshwater sediments (Brinkhurst 1966; Lindegaard and Dall 1988). However, *Tubifex* was found less often than *Limnodrilus* in coarse sediments in natural systems (Lindegaard and Dall 1988). Most studies (Juget 1979; Sauter and Güde 1996) also indicated that *Tubifex* was less adapted than *Limnodrilus* to ingest coarse sediments despite the fact that *Tubifex tubifex* did not exhibit a higher preference for silt–clay (<63 µm) than for coarse sand (500–850 µm) in experimental conditions (Lazim and Learner 1987). Therefore, in comparison with fine sediments, these two genera could act differently in hyporheic habitats because they differ in their ecological requirements. To test the redundancy within the tubificid group, the effects of two worm densities (50 and 100 oligochaetes per column) of *Limnodrilus* and *Tubifex* on ecosystem processes were measured and compared. Four sets of parameters were used to estimate the effect of oligochaetes on system functioning: particle redistribution by the worms (luminophore method), biofilm characteristics (numbers of ETS-active bacteria (with an active electron transport system) and total bacteria), microbial processes (denitrification potential and hydrolytic activity), and water chemistry resulting from microbial processes (NH_4^+, NO_2^-, NO_3^-, dissolved organic carbon (DOC), and oxygen concentrations).

Materials and methods

Sediment preparation

In an 8-L plastic flask, 5.5 kg of calcinated (550°C) sand (grain size 60–630 µm) were combined with 72.5 g of cellulose powder (20 µm) as a source of organic carbon commonly found in natural sediment. The final concentration of particulate organic matter in sand (1.3% of dry sand) corresponded to contents measured in secondary channels of the River Rhône (Mermillod-Blondin et al. 2000*b*). Dechlorinated tap water (2 L) containing 2 mg·L^{-1} DOC and 20 mg·L^{-1} NO_3^- was added to the sand. This sediment was inoculated with an extract of natural bacteria obtained from a pond sediment as in Mermillod-Blondin et al. (2000*a*). A natural extract of bacteria was used to reproduce most bacterial processes occurring in natural systems.

Experimental device

According to the experimental system developed by Danielopol and Niederreiter (in Torreiter et al. 1994 and Griebler 1996), the experiments took place in gravel–sand filtration columns (50-cm height and 10-cm diameter). Each column was filled to a height of 40 cm with calcinated (550°C) fine gravel (4–5 mm) and the sand previously incubated with bacteria during 4 days as explained above. Constant masses of gravel (590 g) and incubated sand (210 g) were alternately added to obtain a heterogeneous interface with presence of zones where sand was trapped and zones with macropores. A water column (6–10 cm) was left at the surface of the sediment to simulate the overlying water of the river. Five centimetres above the sediment surface and 1, 5, 15, and 35 cm below the sediment surface, openings were present in the columns to collect water and fine sediment with a syringe (see details in Mermillod-Blondin et al. 2000*a*).

The experiments were performed in a constant temperature room at 15 ± 0.5°C, and the light was controlled on a 12 h light – 12 h dark cycle in the overlying water. In contrast, the sediment of the column was kept in the dark to suppress photoautotrophic growth. Dechlorinated tap water permanently aerated to keep high oxygen content was continuously fed into the upper part of the four columns with the same peristaltic pump. Before entering the columns, this water was enriched with nitrate (potassium nitrate to obtain a final concentration of 20 mg·L^{-1} NO_3^-) and DOC (2 mg·L^{-1} potassium acetate) with another peristaltic pump. These concentrations of nutrients and DOC were chosen according to values measured in the braided channels of the River Rhône (Bornette and Amoros 1991; Mermillod-Blondin et al. 2000*b*). The resulting infiltration rate in columns was 2.0 ± 0.1 mL·min^{-1} (1.53 cm·h^{-1}). The first measurements were run 3 days after the beginning of the water flow so that the initial hydraulic properties were stabilized.

Experimental design

One experiment consisted of four columns: three columns with the same oligochaete assemblages (oligochaete columns) and one column without oligochaetes used as a control. A previous experiment performed with four control columns showed similar spatial and temporal patterns of solutes and microbes in the four columns during 20 days. No significant differences were measured among the four columns, whereas in all of them, the same significant differences appeared in solute and microbial distributions among depths and dates (Table 1). This low variability between control columns of the same experiment indicated that the use of one control per experiment was acceptable to assess the effects of worms on system functioning.

The four oligochaete treatments were (*i*) 50 individuals of *Limnodrilus* per column, (*ii*) 50 individuals of *Tubifex* per column, (*iii*) 100 individuals of *Limnodrilus* per column, and (*iv*) 100 individuals of *Tubifex* per column. The densities tested were equivalent to 6500 individuals·m^{-2} and 13 000 individuals·m^{-2} and approached tubificid densities of natural coarse sediments observed in Lake Esrom (Lindegaard and Dall 1988) or tubificid densities used in the columns filled with coarse-textured sediments by Chatarpaul et al. (1980). Oligochaetes were obtained from two sites: a dead arm of the Rhône about 20 km upstream of Lyon and the River Semène. All worms without capillary chaeta collected in the dead arm of the Rhône were represented by two species of the *Limnodrilus* genus (80% *Limnodrilus hoffmeisteri* and 20% *Limnodrilus claparedeanus*), whereas all worms collected in the River Semène belonged to the species *Tubifex tubifex*. Therefore, worms collected at these two sites were separated in two groups (*Limnodrilus* and *Tubifex*) without excessive manipulations that can damage the organisms. For acclimatization, the two genera of worms were kept separately under similar experimental conditions (granulometry and food) for more than 15 days before introduction into the columns. The first measurements (day 0) confirmed constency

Table 1. Test of the variability between four control columns of the same experiment (20 days) on chemical and microbial parameters using two-way ANOVA (p values).

Parameters	Tests	Factors	p value
O_2 concentrations	ANOVA column × depth	Column	0.47 (ns)
		Depth	0.0001 (***)
		Interaction	0.58 (ns)
	ANOVA column × date	Column	0.996 (ns)
		Date	0.94 (ns)
		Interaction	0.99 (ns)
Dissolved organic carbon concentrations	ANOVA column × depth	Column	0.63 (ns)
		Depth	0.003 (**)
		Interaction	0.12 (ns)
	ANOVA column × date	Column	0.87 (ns)
		Date	0.043 (*)
		Interaction	0.58 (ns)
NO_3^- concentrations at 35-cm depth	ANOVA column × date	Column	0.95 (ns)
		Date	0.02 (*)
		Interaction	0.996 (ns)
Total number of bacteria	ANOVA column × depth	Column	0.468 (ns)
		Depth	0.0001 (***)
		Interaction	0.335 (ns)
	ANOVA column × date	Column	0.635 (ns)
		Date	0.082 (ns)
		Interaction	0.792 (ns)
Number of ETS-active bacteria	ANOVA column × depth	Column	0.331 (ns)
		Depth	0.01 (*)
		Interaction	0.819 (ns)
	ANOVA column × date	Column	0.223 (ns)
		Date	0.0005 (***)
		Interaction	0.341 (ns)

Note: Significance levels at which the null hypothesis was rejected: ***, $p < 0.001$; **, $p < 0.01$; *, $p < 0.05$; ns, not significant; $n = 4$ for depths (5 cm above and 5 cm, 15 cm, and 35 cm below the sediment surface), and $n = 5$ for dates (days 0, 5, 10, 15, and 20). ETS-active bacteria were bacteria with an active electron transport system.

in conditions among the four columns before the introduction of the tubificid worms. After this control, tubificid worms were introduced into the overlying water of each oligochaete column. To eliminate the variability in worm population from one experiment to another, all tubicid worms used had a biovolume of 4 mm^3 (15- to 20-mm length and 5-mm diameter).

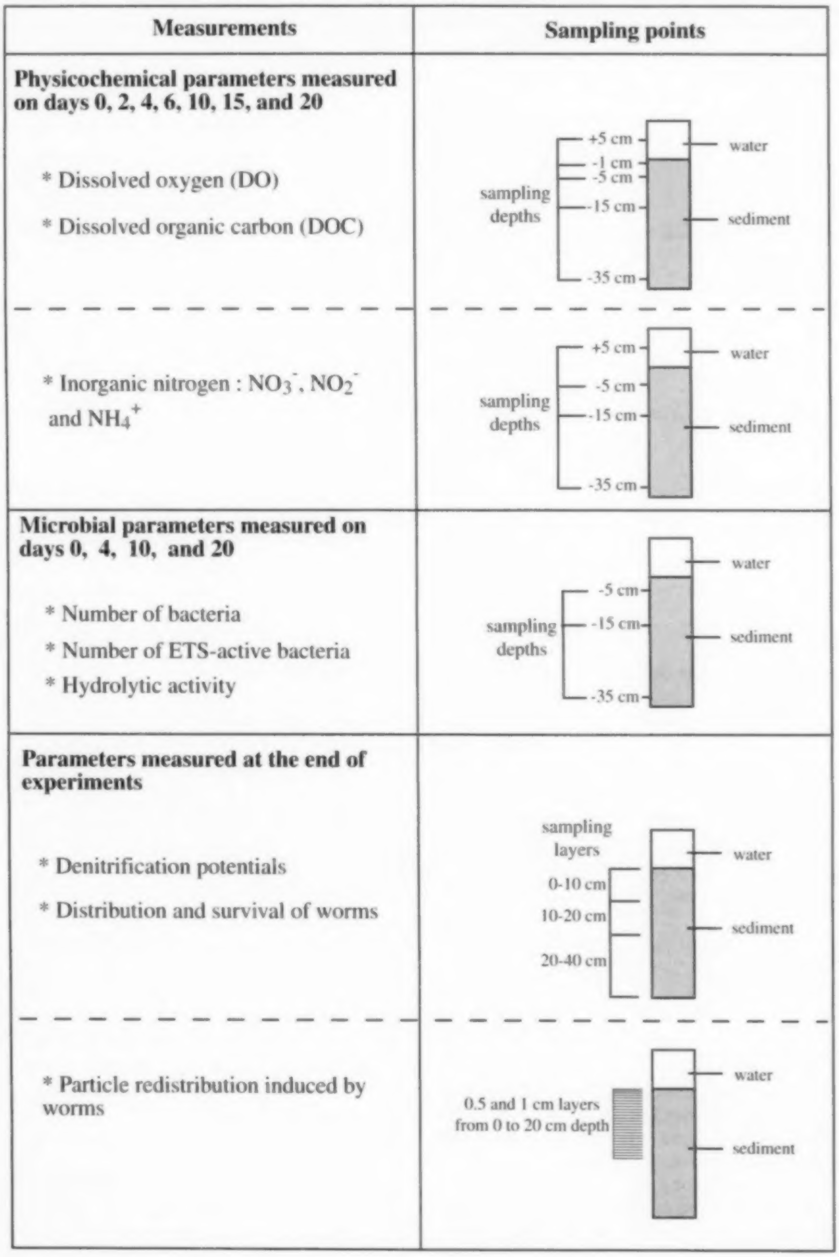
During the 20 days of the experiment, physical, chemical, and bacteriological parameters were measured at different depths and dates in the four columns to evaluate the role of the oligochaetes (Fig. 1). Particle redistribution induced by worms and denitrification potentials of the microorganisms developed on sediment were evaluated at the end of the experiments. Dissolved oxygen (O_2) and DOC concentrations were measured on days 0, 2, 4, 6, 10, 15, and 20 at five depths (5 cm above the sediment surface and 1, 5, 15, and 35 cm below the sediment surface). On the same days, nitrate, nitrite, and ammonium concentrations were measured at four depths (5 cm above the sediment surface and 5, 15, and 35 cm below the sediment surface). Microbial parameters (bacterial abundance, number of ETS-active bacteria, and hydrolytic activity) were measured in the sediment sampled with a syringe at three depths (5, 15, and 35 cm below the sediment surface) on days 0, 4, 10, and 20 of the experiment. These microbial parameters were not measured at a depth of 1 cm because there was not enough sand for analysis at this depth.

According to a previous study (Mermillod-Blondin et al. 2000*a*), NO_3^- concentrations were only presented at the 35-cm depth where they were used to indicate anaerobic processes (denitrification) in the columns. In the same way, DOC results were considered at depths of 1 cm and 35 cm as indicators of aerobic mineralization in surface sediment and anaerobic processes in deeper layers, respectively.

Methods of analysis

Particle redistribution in the columns was quantified by the luminophore tracer technique (Gérino et al. 1994). To reproduce the grain-size heterogeneity of the sand present in the columns, two size fractions of luminophores (natural sediment particles dyed with fluorescent paint) were used: 63- to 100-μm particles (pink) and 100- to 350-μm particles (yellow). These two fractions were cumulated in the results analysis. On day 0 of each experiment, a mixture of the two fractions (0.5 g per fraction) was deposited at the surface of the sediment of the four columns before oligochaete introduction. After a 20-day period, water was evacuated from all columns, and the columns were opened to sample sediment and luminophores. The top 2 cm of each column were collected in 0.5-cm layers, and the next 18 cm were collected in 1-cm layers. Each layer was homogenized and a 1-g subsample was dried at 50°C for luminophore counting. The number of luminophores was estimated under an ultraviolet (UV) light microscope and converted into grams of tracers per gram of dry sediment. The effect of oligochaetes on

Fig. 1. Schematic representation indicating the dates and positions of sampling for each measured parameter.


Measurements
Sampling points
Physicochemical parameters measured on days 0, 2, 4, 6, 10, 15, and 20
* Dissolved oxygen (DO)
* Dissolved organic carbon (DOC)
sampling depths
+5 cm
-1 cm
-5 cm
-15 cm
-35 cm
water
sediment
* Inorganic nitrogen : NO3-, NO2- and NH4+
sampling depths
+5 cm
-5 cm
-15 cm
-35 cm
water
sediment
Microbial parameters measured on days 0, 4, 10, and 20
* Number of bacteria
* Number of ETS-active bacteria
* Hydrolytic activity
sampling depths
-5 cm
-15 cm
-35 cm
water
sediment
Parameters measured at the end of experiments
* Denitrification potentials
* Distribution and survival of worms
sampling layers
0-10 cm
10-20 cm
20-40 cm
water
sediment
* Particle redistribution induced by worms
0.5 and 1 cm layers from 0 to 20 cm depth
water
sediment

particulate matter transport was estimated with a diffusive–advective type transport model (Gérino et al. 1994) adapted to simulate vertical distributions of luminophores. Biological diffusive transport (D_b, cm^2·year^{-1}) was defined as omnidirectional transport in the sediment column, by analogy with molecular diffusion in water. The biodiffusion generates a spreading of tracers in the sediment column (Guinasso and Schink 1975) by exchanges of small amounts of material with adjacent sediment. In control columns with continuous water flowing through the sediment column, tracer migration is due to hydrodynamic effects and gives an estimation of physical diffusion *D*. In fauna columns, the diffusive term is called total diffusion (D_t) and corresponds to the addition of both diffusive transport (D) and biodiffusion (D_b). Vertical bioadvective transport (V_b, cm·year^{-1}) is created by head-down deposit feeders such as tubificid worms that feed in the sediment and defecate pellets at the sediment surface (conveyor-belt organisms). This mode of feeding (called bioadvection) accelerates the rate of sediment and pore water burial within the feeding zone. The global bioadvective coefficient V_t takes into account hydrodynamic effects (V) and bioadvective transport (V_b). The fundamental model equation for tracer distribution in experimental columns is

$$(1)\qquad \frac{\partial C}{\partial t} = D_t\frac{\partial^2 C}{\partial z^2} - V_t\frac{\partial C}{\partial z}$$

where t = time, z = depth, and C = tracer concentration. Luminophores are typically conservative tracers so that the model does not include a reactive term.

Luminophores were added as pulse input at the surface of the sediment at the beginning of each experiment so that the model was applied under non-steady-state conditions. Thus model eq. 1 was used with the upper boundary condition of an instantaneous source of unit strength (maximal C of tracer) at $z = 0$ at $t = 0$, a lower boundary $C \to 0$ at $z \to \infty$, and initial condition $C = 0$ at $z > 0$. The general solution was given by Officer and Lynch (1982) as

$$(2)\qquad c(z,t) = \frac{1}{\sqrt{\pi Dt}}\exp\left(-\frac{(z-Vt)^2}{4Dt}\right) - \frac{V}{2D}\exp\left(\frac{Vz}{2D}\right)\operatorname{erfc}\frac{z+Vt}{\sqrt{4Dt}}$$

where

$$\operatorname{erfc}(x) = 1 - \frac{2}{\sqrt{(\pi)}}\int_0^x e^{-t^2}\,dt$$

and c is a normalized concentration relative to unit input. Transport parameters for luminophores were estimated for each column using a least squares fit of the theoretical profile obtained with eq. 2 to the measured concentration pattern.

Oxygen concentration was measured using a circulating system provided with a Clarke-type electrode connected to an Orbisphere oxymeter (model 3600). This procedure allowed suppression of the contact between the water samples and atmospheric oxygen. Nitrate, nitrite, and ammonium contents were measured using colorimetric Hach methods (Hach Company, Loveland, Co.) after filtration through Whatman glass fiber filters (GF/F type, mesh 0.7 µm).

DOC was measured with a Dohrman DC80 total carbon analyser based on UV-promoted potassium persulfate oxidation (precision ±1%) after removing inorganic carbon with orthophosphoric acid (1 $\mu L \cdot mL^{-1}$) and CO_2 stripping under 10 min of oxygen flow. In case an increase in DOC versus depth was noted in a column, additional analyses by Capillary Ion Analyzer were performed to detect the possible occurrence of volatile fatty acids in the interstitial water outlet of the column (as observed in Mermillod-Blondin et al. 2000*a*).

Total bacteria were estimated by epifluorescence after DAPI staining (4′,6-diamino-2-phenylindole, Porter and Feig 1980). Wet sediment (0.3–0.5 g) was dispersed in 40 mL of filtered (on a 0.2-µm acetate membrane) distilled water and 10 mL of a solution of pyrophosphate (0.002 M, final concentration) by sonication (Bioblock Vibracell 72041, for 180 s, power 2, duty cycle 50%). One millilitre of the suspension was filtered through a Whatman glass fiber filter (GF/D type, mesh 2.7 µm) to eliminate the coarsest particles. Staining was performed by addition of 0.1 mL DAPI solution (100 $\mu g \cdot L^{-1}$) to 1 mL of the filtrate. After 10 min of incubation, the suspension was filtered on a GTBP Millipore membrane (mesh 0.2 µm), which was then washed, air-dried, and mounted in low-fluorescence immersion oil on a microscope glass slide. Bacterial counts were performed using an epifluorescence microscope (Zeiss microscope fitted with a 200-W mercury burner and a Zeiss 365-nm excitation filter). At least 30 randomly selected fields were counted per slide. Results were expressed as numbers of bacteria per gram of dry sediment.

The number of ETS-active bacteria was measured using CTC staining (5-cyano-2,3-ditolyltetrazolium chloride, Rodriguez et al. 1992). Wet sediment (0.3–0.5 g) was incubated with a 1.38 $mg \cdot L^{-1}$ CTC solution (final concentration) for 3 h at 20°C and then sonicated (same sonication as for DAPI with a dilution in 20 mL of pyrophosphate solution). Five millilitres of the suspension was filtered through a Whatman glass fiber filter (GF/D type, mesh 2.7 µm). The filtrate was filtered through a GTBP Millipore membrane (mesh 0.2 µm), which was then washed, air-dried, and mounted in low-fluorescence immersion oil on a microscope glass slide. Bacteria were counted with an epifluorescence microscope fitted with a 200-W mercury burner, a blue (420-nm) excitation filter (Zeiss model BP 390-420), and a 540-nm barrier filter (Zeiss model LP 540). At least 30 randomly selected fields were counted per slide. Results were expressed as numbers of bacteria per gram of dry sediment.

Hydrolytic activity was measured using fluorescein diacetate (FDA) as substrate for hydrolases (Fontvieille et al. 1992). Wet sediment (0.5–1 g) was incubated in 3 mL of phosphate buffer (pH 7.6) with 0.1 mL FDA solution (2 $mg \cdot mL^{-1}$) at 20°C in the dark until the green colour of fluorescein became visible (1 to 3 h). The reaction was stopped by freezing the sediment after addition of 3 mL of mercuric chloride solution (200 $mg \cdot L^{-1}$). The absorbance of this solution at 490 nm was measured after filtration through a acetate membrane (HAWP type, mesh 0.45 µm). Results were expressed as micromoles of hydrolysed $FDA \cdot h^{-1} \cdot g$ dry $weight^{-1}$.

At the end of each experiment, wet sediments in each column were collected from three depths: 0-10 cm, 10–20 cm, and 20–40 cm h. Sediments were then dried at 30°C for 1 week, and sand was separated from gravel by filtration on a 1-mm mesh net. Denitrification potentials were measured according to the method described by Lensi et al. (1985). Dry sand (10 g) was placed in 150 mL flasks and 3 mL of sterilized distilled water containing KNO_3 (0.1 mg $N \cdot g^{-1}$ dry sand), glucose (1 mg $C \cdot g^{-1}$ dry sand), and glutamic acid (1 mg $C \cdot g^{-1}$ dry sand) were added. The mixture was gently homogenized. The flasks were then sealed with rubber stoppers. The air of each flask was evacuated and replaced by a 90:10 $He–C_2H_2$ mixture to provide anaerobic conditions and inhibition of N_2O reductase activity. After incubation at 24°C for 24 h, gas samples were analyzed for N_2O with an electron capture gas-detector chromatograph Varian 3400 Cx. Denitrification potentials were expressed as milligrams of N_2O-N produced per gram of sediment. As a complementary analysis, denitrification potentials were also measured on three particle sizes of the sediment (gravel, coarse sand (>315 µm), and fine sand (<315 µm)) to explain the importance of sediment size on denitrification potentials. This analysis was performed using sediments collected in the control column of the experiment with 100 *Tubifex*. Six replicate samples of each sediment grain size were analysed according to the method previously described (Lensi et al. 1985).

At the end of each experiment, the living worms were counted at three depths (0–10 cm, 10–20 cm, and 20–40 cm) of each fauna column. The number of collected worms was used to estimate worm mortality and also to give information about the vertical distribution of worms in columns.

Data treatment

The effects of each oligochaete treatment were evaluated by comparing values obtained with and without oligochaetes for each experimental set, date, and variable. To describe the effect of oligochaete on processes (sediment fluxes and microbial activities), the use of percentage or ratio gave a direct estimation of stimulation compared to the processes in the control column. For number of bacteria, values were expressed as percentage of the control because a mean difference of 10^3 bacteria could be significant although it only represents 0.01% of bacteria present in control

Fig. 2. Vertical distributions of luminophores obtained in the oligochaete (solid) and control columns (open) of the experiment with 100 *Tubifex*. Values in oligochaete columns are means ± confidence intervals ($n = 3$).

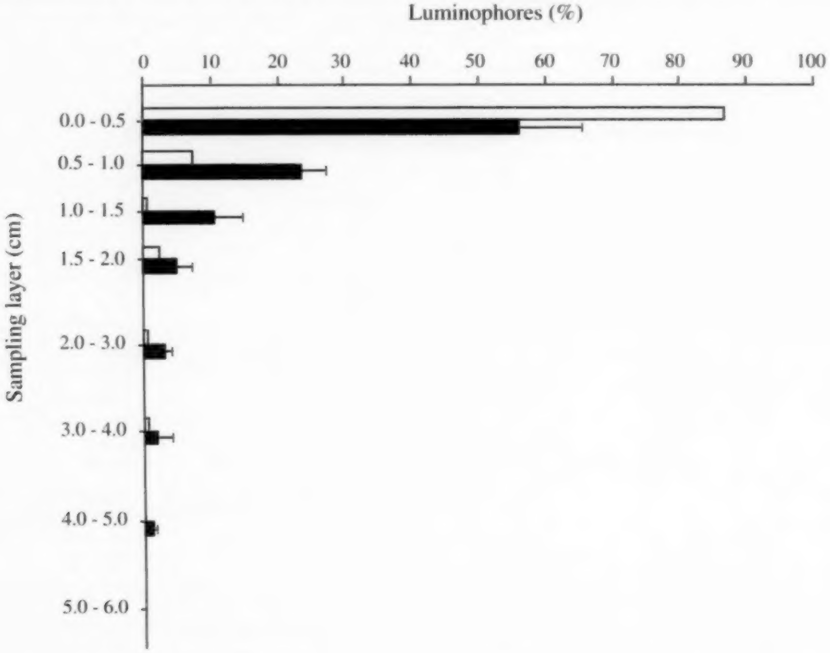


Table 2. Comparison of diffusive mixing rates of sediment estimated in control (D) and oligochaete (D_b) columns with the four oligochaete treatments.

Oligochaete treatment	D_b/D	p value
50 *Limnodrilus*	0.35	0.08 (ns)
50 *Tubifex*	0.71	0.13 (ns)
100 *Limnodrilus*	0.80	0.07 (ns)
100 *Tubifex*	4.0	0.008 (**)

Note: Oligochaete impact on transport intensity is expressed as a ratio of average biodiffusion on simple diffusion measured in the control of the same experiment. ns, not significant; *, $p < 0.05$; **, $p < 0.01$.

columns (10^7 bacteria). In contrast, we used differences rather than percentages for chemical variables because the change expressed as a percentage was highly dependent on the control value. For example, a difference of 20% in O_2 concentration did not have the same significance with 2.0 $mg \cdot L^{-1}$ O_2 in control (difference of 0.4 $mg \cdot L^{-1}$) as with 5 $mg \cdot L^{-1}$ O_2 in the control (difference of 1 $mg \cdot L^{-1}$). The impact of oligochaete was clearly higher (250%) in the second case.

Therefore, oligochaete effects on particle distribution were estimated at the end of each experiment by the ratio between mean particle flux in oligochaete columns and the flux measured in the control column ($n = 3$, differences between three oligochaete columns and the control column of the same experiment). Microbial parameters were rapidly modified by tubificid worms, and significant effects were measured on day 4 of the experiment (as observed in Mermillod-Blondin et al. 2000*a*). Therefore, worm effects on microbial characteristics were estimated by calculating the average difference between oligochaete and control columns (expressed as a percentage of the control values) measured on days 4, 10, and 20 ($n = 9$, differences between three oligochaete columns and the control column of the same experiment at three dates). This analysis indicated the mean effect of each worm treatment on microbial parameters at each depth during the experiment. Despite a rapid modification of microbial parameters by the worms, the impact of oligochaetes on O_2, DOC, and NO_3^- concentrations was observed only after 10 days of the experiment (as observed in Mermillod-Blondin et al. 2000*a*). Therefore, the effect of oligochaetes on these solutes was estimated on the last two sampling dates when both aerobic and anaerobic processes were modified by the worms. For these variables, we calculated the average differences between oligochaete and control columns measured on days 15 and 20 at each depth ($n = 6$, differences between three oligochaete columns and the control column of the same experiment at two dates).

For each experimental set, parametric t tests (in comparison to the theoretical value of 0, Sokal and Rohlf 1969) were performed on the differences calculated between the oligochaete column data and the control column data for each depth and each variable. This analysis made it possible to test the null hypothesis that oligochaete treatment did not create changes in sediment redistribution, nutrient concentrations, and microbial distributions. When $p < 0.05$, differences were considered significant and were indicated in the figures with an asterisk (*). Parameters expressed as percentages (sediment fluxes and microbial parameters) were arcsine transformed (Sokal and Rohlf 1969). To test the difference between the effects of *Limnodrilus* and those of *Tubifex*, parameters expressed as ratios (particle fluxes), differences (chemical parameters), and percentages of the difference (microbial parameters) between the fauna and control columns were compared using the parametric t test for each density (comparison of the effects of *Limnodrilus* and those of *Tubifex* at both densities). Denitrification potentials obtained from three sediment grain sizes were compared using a one-way analysis of variance (ANOVA). Subsequently, Fisher's PLSD (protected least significant difference) comparisons were used to evaluate differences in denitrification potentials between each sediment grain size. Data on number of living worms found at the end of experiments were expressed as percentages and

Table 3. Average values of O_2 concentrations ($mg \cdot L^{-1}$) measured during days 15 and 20 at the five depths in control and oligochaete columns for the four experiments.

	Oligochaete treatment							
	50 *Limnodrilus*		50 *Tubifex*		100 *Limnodrilus*		100 *Tubifex*	
Sampling depth	Control	With fauna	Control	With fauna	Control	With fauna	Control	With fauna
+5 cm	5.7 (0.39)	5.37 (0.26)	5.15 (0.49)	4.97 (0.17)	5.55 (0.49)	5.72 (0.16)	5.95 (0.10)	5.77 (0.07)
–1 cm	3.7 (0.05)	3.15 (0.42)	3.4 (0.20)	2.7 (0.26)	3.95 (0.49)	3.48 (0.18)	3.3 (0.20)	2.93 (0.11)
–5 cm	2.5 (0.20)	2.32 (0.14)	2.85 (0.29)	2.12 (0.06)	2.6 (0.20)	2.0 (0.16)	2.7 (0.20)	2.02 (0.12)
–15 cm	2.0 (0.05)	1.9 (0.07)	2.1 (0.05)	1.95 (0.08)	1.95 (0.10)	1.38 (0.15)	2.05 (0.10)	1.72 (0.09)
–35 cm	1.85 (0.10)	1.78 (0.06)	1.85 (0.10)	1.67 (0.11)	1.67 (0.11)	1.17 (0.07)	1.95 (0.10)	1.55 (0.08)

Note: Values in parentheses are confidence intervals.

were arcsine transformed. The percentage of living worms was compared between oligochaete treatments using a one-way ANOVA. The numbers of worms found at the three sampling layers were also expressed as percentages and were arcsine transformed. The effects of depth and oligochaete treatment on worm distributions were tested using a two-way ANOVA (depth × experiment). Statistical analyses were performed using Statview F-5.0 (SAS Institute Inc., Cary, N.C.).

Results

Effect of worms on particle redistribution

Tracer distributions in control and all fauna columns exhibited an exponential decrease with sediment depth typical of diffusion-like transport without any occurrence of advection processes (as in the experiment with 100 *Tubifex*, Fig. 2). A small quantity of the tracer was found below 1.5 cm in the control while the tracer occasionally migrated 4 cm down into the sediment when oligochaetes were present (Fig. 2). Biological change expressed as the ratio between the average diffusion coefficients ($n = 3$) estimated with oligochaetes and the diffusion coefficient estimated in the control column of the same experiment are indicated in Table 2. Fifty *Limnodrilus* or 50 *Tubifex* per column did not significantly modify surface particle distribution. However, 100 *Tubifex* per column significantly increased the diffusive particle transport, whereas 100 *Limnodrilus* had no effect on particle distribution.

Effects of worms on O_2 contents

In all columns, DOC showed a sharp drop with depth, but O_2 concentration at 35 cm was always higher than 1 $mg \cdot L^{-1}$ (Table 3). All oligochaete treatments significantly decreased O_2 concentrations at all depths in the sediment (Fig. 3). The differences in O_2 concentrations were greatest in the first 5 cm of the sediment. At 15 cm and 35 cm, these differences were lower except with 100 *Limnodrilus*. With a density of 50 worms per column, no significant differences were measured between the effects of *Limnodrilus* and those of *Tubifex* on O_2 concentration at depths of 1, 15, and 35 cm. However, a significantly higher O_2 depletion was measured with *Tubifex* than with *Limnodrilus* at 5 cm ($p < 0.001$, t test). With a density of 100 worms per column, the effects of *Tubifex* and *Limnodrilus* on O_2 concentration were not significantly different at 1 and 5 cm in the sediment. However, at depths of 15 and 35 cm, *Limnodrilus* produced a higher O_2 depletion than *Tubifex* ($p < 0.05$, t test).

Effect of worms on DOC and NO_3^- contents

At 1 cm, 50 *Tubifex* had a significantly different effect on DOC concentration, whereas 50 *Limnodrilus* did not(Fig. 4*a*, $p < 0.05$, t test). The presence of *Tubifex* produced lower DOC concentration than in the control, whereas the presence of *Limnodrilus* had no effect on DOC concentration. In contrast, both oligochaete treatments with 100 tubificid worms significantly decreased DOC concentration at 1 cm. At 35 cm, despite no significant effect resulting from the presence of 50 and 100 *Limnodrilus* per column, DOC concentrations tended to increase in all experiments with the same intensity (Fig. 4*b*). Complementary analyses indicated that this DOC production was due to volatile fatty acids (malic, acetic, and propionic acids). At the two densities, *Tubifex* and *Limnodrilus* did not produce any significantly different effect ($p > 0.5$, t tests).

In contrast with DOC concentrations, the concentration of NO_3^- at 35 cm decreased with most oligochaete treatments (Fig. 4*c*). No significant differences in decrease of NO_3^- concentration were measured between the treatments with 50 *Limnodrilus* and 50 *Tubifex* per column ($p > 0.2$, t test), and the same result occurred with 100 individuals of each genus ($p > 0.5$, t test).

Effect of worms on microbial parameters

With 50 worms per column, *Tubifex* significantly increased the total number of bacteria at 5 and 35 cm, whereas *Limnodrilus* had no significant effect on this parameter (Fig. 5*a*). However, there were no significant differences between the effects of the two genera on total numbers of bacteria at all depths ($p > 0.1$, t tests). At a density of 100 worms, *Limnodrilus* and *Tubifex* had similar effects on bacterial abundance at the three depths ($p > 0.5$, t tests) despite the fact that the worm effects were not always significant in comparison with the control (*Tubifex* at 5 and 15 cm).

With 50 worms per column, *Limnodrilus* and *Tubifex* produced significantly different changes on number of ETS-active bacteria at depths of 5 ($p < 0.03$, t test) and 15 cm ($p < 0.01$, t test). *Tubifex* sp. significantly increased the number of ETS-active bacteria at 5 cm, whereas *Limnodrilus* had no effect (Fig. 5*b*). In contrast, at 15 cm, *Tubifex* had a negative effect on the number of ETS-active bacteria, whereas *Limnodrilus* increased, but not significantly, this parameter. At 35 cm, *Limnodrilus* and *Tubifex* did not significantly modify the number of ETS-active bacteria. At a density of 100 worms, the two genera created a similar and significant increase in numbers of ETS-active bacteria at depths of 5 and 15 cm ($p > 0.05$, t tests). However, at 35 cm, the two

Fig. 3. Differences in O_2 concentrations ($mg \cdot L^{-1}$) between fauna and control columns for each oligochaete treatment (open, 50 *Limnodrilus*; solid, 50 *Tubifex*; shaded, 100 *Limnodrilus*; cross-hatched, 100 *Tubifex*) at (*a*) 1 cm, (*b*) 5 cm, (*c*) 15 cm, and (*d*) 35 cm below the sediment surface. Values are means ± confidence intervals ($n = 6$). * indicates a significant difference between control and oligochaete columns.

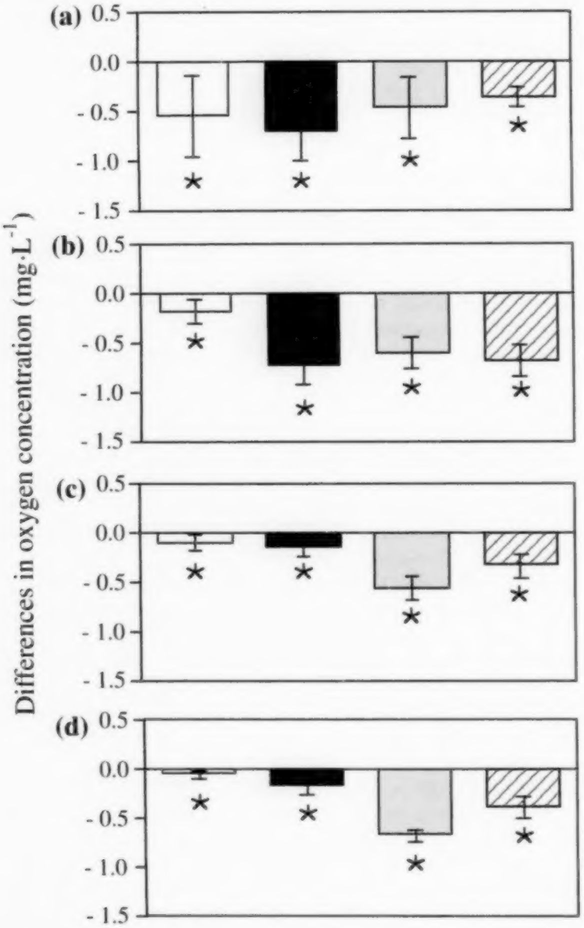
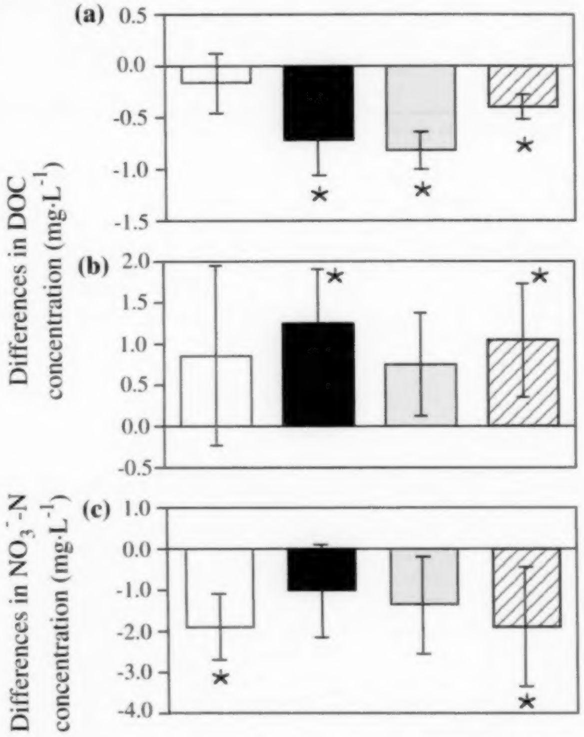


Fig. 4. Differences in DOC at (*a*) 1 cm and (*b*) 35 cm and in (*c*) NO_3^--N concentrations at 35 cm between fauna and control columns for each oligochaete treatment (open, 50 *Limnodrilus*; solid, 50 *Tubifex*; shaded, 100 *Limnodrilus*; cross-hatched, 100 *Tubifex*). Values are means ± confidence intervals ($n = 6$). * indicates a significant difference between control and oligochaete columns.



genera had significantly different effects on active bacteria ($p < 0.05$, *t* test). *Limnodrilus* significantly increased the number of active bacteria, whereas the 100 *Tubifex* had no effect on this parameter.

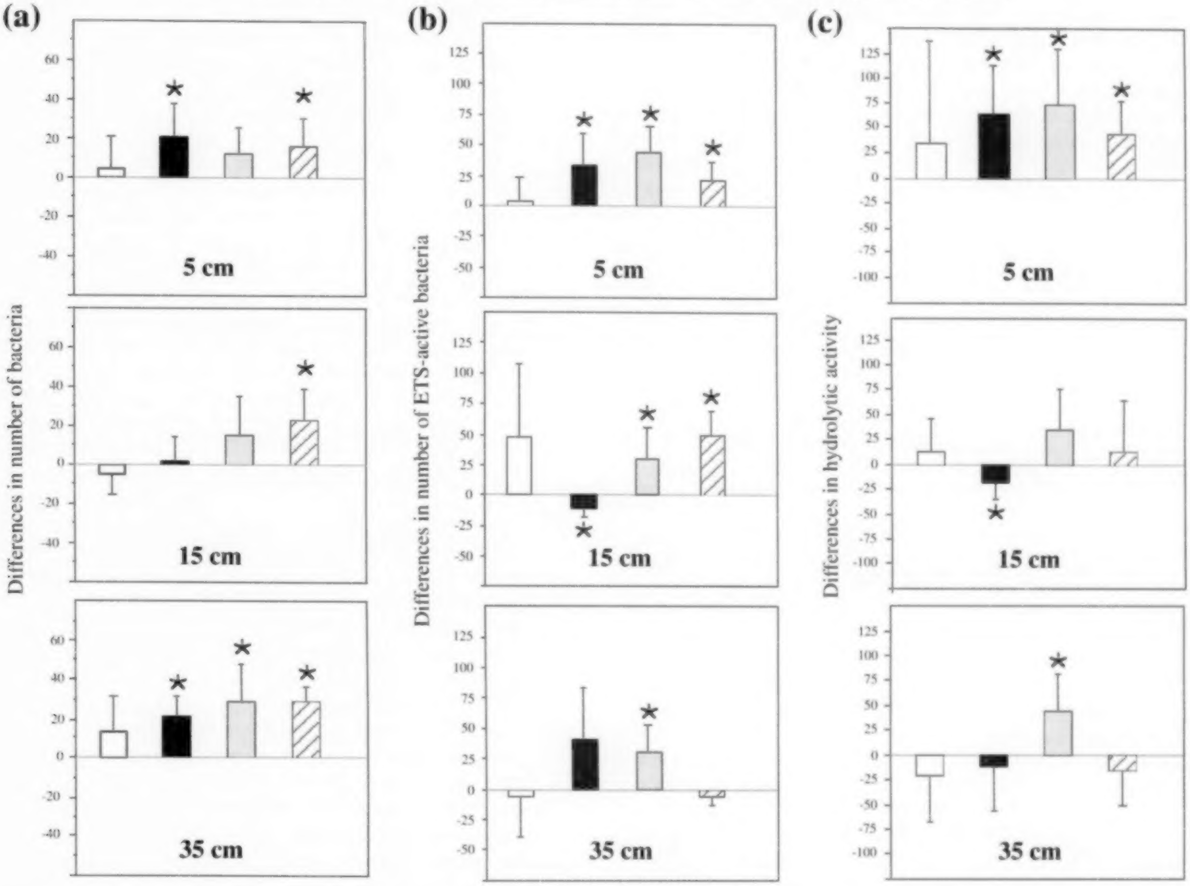
All oligochaete treatments stimulated hydrolytic activity at 5 cm below the sediment surface except the 50 individuals of *Limnodrilus* (Fig. 5*c*). With 50 oligochaetes per column, despite no significant differences between the two genera at the three depths ($p > 0.15$, *t* tests), *Limnodrilus* did not significantly modify the hydrolytic activity, whereas *Tubifex* had a positive effect at 5 cm and a negative effect at 15 cm. With a density of 100 oligochaetes, *Limnodrilus* and *Tubifex* did not produce significantly different changes in hydrolytic activity except at 35 cm ($p < 0.02$, *t* test), where only *Limnodrilus* had a positive and significant effect.

The differences in denitrification potentials resulting from oligochaete treatments are shown in Table 4. Although the presence of worms significantly modified the denitrification potentials in three cases, no clear effect of all oligochaete treatments was observed owing to a high heterogeneity. The denitrification potentials measured on three particle sizes (gravel, coarse sand (>315 μm), and fine sand (<315 μm)) were clearly higher in fine sand (22.9 ± 3.6 mg $N_2O\text{-}N \cdot g^{-1}$ sediment) than in the other two fractions ($p < 0.001$, one-way ANOVA), which showed no significantly different values (3.6 ± 0.4 mg $N_2O\text{-}N \cdot g^{-1}$ sediment in the coarse sand and 4.1 ± 1.2 mg $N_2O\text{-}N \cdot g^{-1}$ sediment in the gravel ($p = 0.72$, Fisher's PLSD)).

Estimated mortality and distribution of worms at the end of the experiments

Whatever the experiment, the percentage of living worms varied from 79% to 90% (Table 5) and was not significantly different among experiments ($p = 0.86$, one-way ANOVA). The number of worms found at each layer of sediment showed that most worms (60–86%) were observed in the first 10 cm of sediment ($p < 0.001$, depth effect, two-way ANOVA). Furthermore, only individuals of *Limnodrilus* were found in the deeper layer of sediment at the end of the experiment using 100 *Limnodrilus* (Table 5). However, for

Fig. 5. Differences between fauna and control columns (expressed as percentages of the controls) in numbers of (*a*) bacteria and (*b*) bacteria with an active electron transport system (ETS-active bacteria) and (*c*) hydrolytic activity at three depths for each oligochaete treatment (open, 50 *Limnodrilus*; solid, 50 *Tubifex*; shaded, 100 *Limnodrilus*; cross-hatched, 100 *Tubifex*). Values are means ± confidence intervals (n = 9). * indicates a significant difference between control and oligochaete columns.



the two tested densities of worms, no significant differences were measured between the vertical distribution of *Limnodrilus* and that of *Tubifex* ($p > 0.98$, experiment effect, two-way ANOVA).

Discussion

Our results show that oligochaete effects on sediment redistribution, aerobic respiration, denitrification, and organic matter mineralization depend on the worm populations. However, before interpreting worm effects, the physical characteristics of the system must be briefly discussed and linked to the biogeochemical functioning.

Physical heterogeneity of the system

As observed in a previous study (Mermillod-Blondin et al. 2000*a*), the occurrence of denitrification (NO_3^- decrease) and fermentative processes (production of volatile fatty acids resulting from the anaerobic degradation of the cellulose powder) at O_2 concentrations (mean values measured at each sampling depth) always above 1.0 mg·L^{-1} indicated that anaerobic processes occurred locally in microenvironment. The high physical and chemical (solution and solid phase) heterogeneity of the porous medium controlled the local availability of nutrients, which led to a heterogeneous distribution of microbial activities (as in Murphy et al. 1997). For example, the strong differences in denitrification potentials measured between three sediments with different particle sizes was in accordance with other studies (Dodds et al. 1996; Griebler 1996) and indicated a preponderant effect of particle size and particle distribution on microbial colonization and the occurrence of aerobic and anaerobic microenvironments. Thus, the experimental system presented a physical heterogeneity similar to that observed in studies on the hyporheic zone (Danielopol 1989; Triska et al. 1993; Mermillod-Blondin et al. 2000*b*).

Functional redundancy of *Limnodrilus* and *Tubifex*

The hypothesis that the representatives of the two genera were redundant in coarse sediments was validated with respect to most of the measures used to assess their impact on the system. *Limnodrilus* and *Tubifex* clearly produced the same kind of change on chemical parameters resulting from

Table 4. Differences in denitrification potential between oligochaete treatment and control (expressed as percentages) for each experiment at three layers of sediment.

Oligochaete treatment	Sampling layers (cm)	Mean differences	95% CI	Oligochaete treatment vs. control *p* value
50 *Limnodrilus*	0–10	–34.31	13.11	0.036 (*)
	10–20	–10.31	46.62	0.707 (ns)
	20–40	–45.72	68.56	0.321 (ns)
50 *Tubifex*	0–10	934.56	187.09	0.001 (**)
	10–20	138.39	289.04	0.447 (ns)
	20–40	–10.67	68.21	0.788 (ns)
100 *Limnodrilus*	0–10	88.33	84.87	0.177 (ns)
	10–20	28.33	54.69	0.447 (ns)
	20–40	152.86	134.18	0.157 (ns)
100 *Tubifex*	0–10	15.70	23.02	0.313 (ns)
	10–20	–32.30	46.84	0.309 (ns)
	20–40	616.63	58.8	0.002 (**)

Note: Mean differences and confidence intervals (CI) of differences ($n = 3$). Results of Student's *t* tests performed to compare oligochaete treatment and control are indicated in the last column. ns, not significant; *, $p < 0.05$; **, $p < 0.01$.

Table 5. Numbers of living worms found at the end of the four experiments in oligochaete columns at three sediment layers.

	Oligochaete treatment			
	50 *Limnodrilus*	50 *Tubifex*	100 *Limnodrulus*	100 *Tubifex*
Oligochaete column 1				
0–10 cm	41	39	61	74
10–20 cm	3	2	16	8
20–40 cm	0	0	2	0
% of living worms	88	82	79	82
Oligochaete column 2				
0–10 cm	35	38	77	76
10–20 cm	5	4	8	9
20–40 cm	0	0	0	0
% of living worms	80	84	85	85
Oligochaete column 3				
0–10 cm	40	43	82	77
10–20 cm	0	1	6	4
20–40 cm	0	0	2	0
% of living worms	80	88	90	81

Note: Estimated percentages of living worms were indicated for each fauna column.

microbial processes. As observed by many authors in other aquatic systems (Chatarpaul et al. 1980; Torreiter et al. 1994; van de Bund et al. 1994), tubificid worms played a significant role in the processing of organic matter both in aerobic and anaerobic conditions. Most measurements (oxygen concentrations, number of bacteria, and hydrolytic activity) indicated that 100 *Limnodrilus* and 100 *Tubifex* did not produce any significantly different increase in microbial activity in the first 5 cm of the sediment. At the 35-cm depth, the results of NO_3^- and DOC measurements showed that *Limnodrilus* and *Tubifex* also had no significantly different effects on anaerobic processes.

As observed in a previous study (Mermillod-Blondin et al. 2000*a*), O_2 concentrations rapidly declined with sediment depth, presumably owing to microbial respiration. Columns with oligochaetes showed significantly higher uptakes of O_2 in the sediment than did control columns. As the respiration of the worms can only explain less than 10% of the maximum effect of each oligochaete treatment on O_2 concentration (McCall and Fisher 1980), higher O_2 uptakes in oligochaete columns undoubtedly resulted from interactions between oligochaetes and bacteria. The greater effect of most worm treatments on O_2 concentrations at 1-cm and 5-cm depths in comparison with 15-cm and 35-cm depths (except in the experiment with 100 *Limnodrilus*) suggests that the two genera of worms modified predominantly the functioning of the first 5 cm of the sediment. By stimulating microbial respiration, tubificid worms created a difference in O_2 concentration between control and oligochaete columns in the upper layer. This difference was smaller deeper in the sediment because respiration was lower in oligochaete columns than in control columns. In the upper layer of the control columns, O_2 and DOC concentrations were greater than in the oligochaete columns, thus allowing a higher microbial respiration in deeper layers. The higher impact of tubificid worms in the upper layer of the sediment was confirmed by the high percentage of worms found in the 0- to 10-cm layer at the end of the experiments (more than 75%), indicating that worm activities were concentrated in the first 10 cm of the sediment.

The functional redundancy of the representatives of the two genera was certainly due to the fact that *Limnodrilus* and *Tubifex* have similar feeding and bioturbation activities in the coarse sediments of the hyporheic system. As indicated by luminophore profiles, there was no advective transport of luminophores in the sediment owing to oligochaete activity or downward water flow. In contrast, tubificid worms produce a bioadvection (i.e., sediment mixing resulting from particle ingestion within the sediment and egestion at the surface) of surface particles in the fine sediments of lakes (McCall and Tevesz 1982). This biologically induced sediment transport was certainly not measured in our experimental system because most sediment grains were too coarse to be ingested (>200 µm, Juget 1979) by the tested worms. In these conditions, we can suppose that tubificid worms fed selectively on fine particles (cellulose powder) in experimental columns, according to the findings of Rodriguez et al. (2000) for *Tubifex tubifex*. Although biologically mediated sediment fluxes were not intense owing to the low fine sediment fraction in the column, 100 *Tubifex* significantly increased the diffusive transport of luminophores in columns. This sediment transport might be due to the presence of tubes and burrows observed in sandy zones presents on inner wall of the columns in all oligochaete experiments. These structures may act as supplementary pathways that facilitated surface tracer propagation in the sediment column (diffusive transport of luminophores). However, if the two genera produced tubes and burrows, that 100 *Tubifex* had a significant effect on luminophore transport whereas 100 *Limnodrilus* did not suggested differences in depth burrowing between the two groups of worms (discussed below).

By feeding on fine particles and associated microflora, the oligochaetes kept the biofilm in an active physiological state, stimulating the microbial activity. Tubificid worms are well known to be head-down deposit feeders that defecate pellets with lower numbers of bacteria at the sediment surface (Wavre and Brinkhurst 1971). Despite low content of fine sediment in the experimental conditions, faecal pellets were observed at the sediment surface, indicating that worms could increase the colonizable area for bacteria by modifying sediment structure at the water–sediment interface. Furthermore, the continuous creation of new burrows and pathways also modified the dispersion of the water flux in the sediment. By increasing the flux dispersion, oligochaetes enhanced the nutrient availability in all microenvironments of the columns, stimulating both aerobic and anaerobic microbial activities. Therefore, the stimulation of anaerobic processes in the deeper layers was not only linked to the low oxygen concentrations induced by oligochaetes but it could also be due to a better water flow dispersion through the burrows. Burrowing by oligochaetes favoured the introduction of NO_3^- into new and deeper reactive niches, as those described by Murphy et al. (1997), where denitrification occurred. This effect was clearly exemplified by the presence of 50 *Limnodrilus*, which decreased NO_3^- concentration at the 35-cm depth but had a low impact on oxygen concentration at the same depth (O_2 depletion owing to 50 *Limnodrilus* was 0.07 mg·L^{-1}). Furthermore, if potential denitrification was not significantly modified by oligochaetes, these worms clearly modified the expression of this potentiality in the columns. Thus, oligochaete activities such as biofilm feeding, faecal pellet production, and new pathway creation should all produce a stimulation of microbial processes. The particle redistribution may also increase microbial activity in the deeper strata of the sediment by increasing the quantity of particulate organic matter.

Death of worms in the columns was estimated at the end of the experiments as 10–21% of the fauna introduced. Decomposition of worms could serve as carbon for bacteria and might increase microbial processes. However, no difference in DOC production was observed at the 35-cm depth between oligochaete treatments, whereas twofold higher numbers of worms did not survive in experiments with 100 individuals (mean = 16 dead worms per column) in comparison with experiments with 50 individuals (mean = 8.2 dead worms per column). This result indicates that DOC was not linked to the number of dead worms, suggesting that the decay of few individuals had no significant effect on microbial processes.

Despite a functional redundancy observed for most consequences of their biological activities, some dissimilarity was observed between the effects of the two genera of tubificid worms. With a density of 50 oligochaetes per column, *Tubifex* produced a higher increase in aerobic microbial activity than did *Limnodrilus* in the first 5 cm of the sediment columns. This difference between worm activities could be linked to the water temperature of 15°C used in the experiments. Several studies (see review in McCall and Tevesz 1982) showed that the feeding rates of three species of worms were highly dependant on temperature. The feeding rate of *Tubifex tubifex* was found to be four times higher than that of *Limnodrilus hoffmeisteri* at 15°C, our experimental temperature. Therefore, that the effect of 50 *Tubifex* was higher than that of 50 *Limnodrilus* in our hyporheic system could be due to different optimum temperatures of the two genera. However, this result is not contrary to a redundancy of the two genera because two members of a same functional group produce effects of the same nature on ecosystem processes but with a possible difference in effect intensity (François et al. 1999).

At a density of 100 individuals per column, *Limnodrilus* and *Tubifex* modified the same environmental parameters but at different locations and with different intensities. *Limnodrilus* induced a greater O_2 depletion and a greater increase of biofilm parameters (bacterial activities) at the 35-cm depth. In contrast, the luminophore results showed that *Tubifex* induced greater changes in the top 5 cm than did *Limnodrilus* by producing significant particle redistribution. As observed by Juget (1984) in the alluvial plain of the French Upper Rhône, these results seem to indicate that *Limnodrilus* burrow deeper than *Tubifex* into the sediment in the experimental system. Despite no significant differences between *Limnodrilus* and *Tubifex*, only individuals of *Limnodrilus* were found in the deeper layer of the sediment at the end of experiments, suggesting a deeper penetration of this genus in coarse sediments. In our experimental conditions, the low fraction of grains smaller than 63 µm could explain the better use of the sediment by *Limnodrilus* because this genus is more tolerant than *Tubifex* of low contents of clay and silt in the sediments (Sauter and Güde 1996). Juget (1979) reported that *Limnodrilus hoffmeisteri* could ingest a larger range of grain sizes than *Tubifex tubifex*. However, the differences in colonized depths between the two genera could also be due to other factors, such as differences in musculature. Juget (University of Lyon, Lyon, France, personal communication) found that *Limnodrilus hoffmeisteri* had a greater musculature (ratio between the longitudinal muscle diameter and animal diameter of 7.8 ± 1.5%) than *Tubifex tubifex* (4.3 ± 1.2%). Therefore, *Limnodrilus* might present a better adaptation to live deeper in the sediment than *Tubifex*. Further investigations would be useful to determine the relative importance of each factor on the differences in worm activity distributions.

In a laboratory experiment, François et al. (1999) measured differences in the intensity of sediment reworking between two bivalves of the same functional group. They pointed out that the specific variability within functional groups is crucial to link diversity and ecosystem function. The differences observed between *Limnodrilus* and *Tubifex* in the present study also indicate that the functional diversity within the functional group of tubificid worms cannot be neglected. For example, in natural systems, the differences in particle redistribution intensities between the two genera can have great consequences on system function because the hyporheic zone depends on the supply of organic matter from the surface (Danielopol 1989). Therefore, the two genera of tubificids function differently at the interface by modifying the transfer of organic matter into the hyporheic system (Gilbert et al. 1997). The present study also indicates that the functional redundancy of two species (or genera) cannot be observed in all ecosystem processes. In a sense, each species (or genus) has its functional peculiarity in an ecosystem.

Acknowledgments

This work was supported by grants from the French National Centre for Scientific Research (No. 98N62/0144, *Programme Environnement Vie et Sociétés*) within the programme "Biodiversité et Fonctionnement des ecosystèmes (Programme National Dynamique de la Biodiversité et Environnement)" and by grants from the Institut Fédératif de Recherche No. 41 (Institut des Sciences et Méthodes de l'Ecologie et de l'Evolution). We thank N. Salin and G. Fauvet for their technical help in laboratory analysis, J. Juget for helpful comments, and F. Malard and E. Pattee for reviewing a earlier draft of this text. We also acknowledge two anonymous referees for commenting on an earlier draft and giving some valuable suggestions that improved the text.

References

Bornette, G., and Amoros, C. 1991. Aquatic vegetation and hydrology of a braided river floodplain. J. Veg. Sci. **2**: 497–512.

Boulton, A.J. 2000. The functional role of the hyporheos. Verh. Int. Verein. Limnol. **27**: 51–63.

Brinkhurst, R.O. 1966. The Tubificidae (Oligochaeta) of polluted waters. Verh. Int. Verein. Limnol. **16**: 54–859.

Chapin, F.S., Schulze, E.-D., and Mooney, H.A. 1992. Biodiversity and ecosystem processes. Trends Ecol. Evol. **7**: 107–108.

Chatarpaul, L., Robinson, J.B., and Kaushik, N.K. 1980. Effects of tubificid worms on denitrification and nitrification in stream sediment. Can. J. Fish. Aquat. Sci. **37**: 656–663.

Danielopol, D.L. 1989. Groundwater fauna associated with riverine aquifers. J. N. Am. Benthol. Soc. **8**: 18–35.

Dodds, W.K., Randel, C.A., and Edler, C.C. 1996. Microcosms for aquifer research: application to colonization of various sized particles by ground-water microorganisms. Ground Water, **34**: 756–759.

Fontvieille, D., Outaguereouine, A., and Thevenot, D.R. 1992. Fluorescein diacetate hydrolysis as a measure of microbial activity in aquatic systems. Application to activated sludge. Environ. Technol. **13**: 531–540.

François, F., Dalègre, K., Gilbert, F., and Stora, G. 1999. Variabilité spécifique à l'intérieur des groupes fonctionnels. Etude du remaniement sédimentaire de deux bivalves Veneridae, *Ruditapes decussatus* et *Venerupis aurea*. C. R. Acad. Sci. Paris, **322**: 339–345.

Gérino, M., Stora, G., and Durbec, J.-P. 1994. Quantitative estimation of biodiffusive and bioadvective sediment mixing: in situ experimental approach. Oceanol. Acta, **17**: 547–554.

Gilbert, F., Stora, G., Bonin, P., LeDréau, Y., Mille, G., and Bertrand, J.-C. 1997. Hydrocarbon influence on denitrification in bioturbated Mediterranean coastal sediments. Hydrobiologia, **345**: 67–77.

Griebler, C. 1996. Some applications for the DMSO-reduction method as a new tool to determine the microbial activity in water-saturated sediments. Arch. Hydrobiol. Suppl. **113**: 405–410.

Guinasso, N.L., Jr, and Schink, D.R. 1975. Quantitative estimates of biological mixing rates in abyssal sediments. Geophys. Res. **80**: 3032–3043.

Juget, J. 1979. La texture granulométrique des sédiments et le régime alimentaire des oligochètes limnicoles. Hydrobiologia, **65**: 145–154.

Juget, J. 1984. Oligochaeta of the epigean and underground fauna of the alluvial plain of the French Upper Rhône (biotypological trial). Hydrobiologia, **115**: 175–182.

Lawton, J.H. 1994. What do species do in ecosystems ? Oikos, **71**: 367–374.

Lazim, M.N., and Learner, M.A. 1987. The influence of sediment composition and leaf litter on the distribution of tubificid worms (Oligochaeta). Oecologia, **72**: 131–136.

Lensi, R., Gourbière, F., and Josserand, A. 1985. Measurement of small amounts of nitrate in an acid soil by N_2O production. Soil Biol. Biochem. **17**: 733–734.

Lindegaard, C., and Dall, P.C. 1988. Abundance and distribution of Oligochaeta in the exposed littoral zone of lake Esrom, Denmark. Arch. Hydrobiol. Suppl. **81**: 533–562.

McCall, P.L., and Fisher, J.B. 1980. Effects of tubificid oligochaetes on physical and chemical properties of Lake Erie sediments. *In* Aquatic oligochaete biology. *Edited by* R.O. Brinkhurst and D.G. Cook. Plenum Press, New York. pp. 253–317.

McCall, P.L., and Tevesz, M.J.S. 1982. The effects of benthos on physical properties of freshwater sediments. *In* Animal–sediment relations. The biogenic alteration of sediments. *Edited by* P.L. McCall and M.J.S. Tevesz. Plenum Press, New York. pp. 105–176.

Mermillod-Blondin, F., Creuzé des Châtelliers, M., Gérino, M., and Gaudet, J.-P. 2000*a*. Testing the effect of *Limnodrilus sp.* (Oligochaeta, Tubificidae) on organic matter and nutrient processing in the hyporheic zone: a microcosm method. Arch. Hydrobiol. **149**: 467–487.

Mermillod-Blondin, F., Creuzé des Châtelliers, M, Marmonier, P., and Dole-Olivier, M.-J. 2000*b*. Distribution of solutes, microbes and invertebrates in river sediments along a riffle–pool–riffle sequence. Freshwater Biol. **44**: 255–269.

Murphy, E.M., Ginn, T.R., Chilakapati, A., Resch, C.T., Phillips, J.L., Wietsma, T.W., and Spadoni, C.M. 1997. The influence of physical heterogeneity on microbial degradation and distribution in porous media. Water Resour. Res. **33**: 1087–1103.

Officer, C.B., and Lynch, D.R. 1982. Interpretation procedures for the determination of sediment parameters from time-dependent flux inputs. Earth Planet. Sci. Lett. **61**: 55–62.

Palmer, M.A., Covich, A.P., Finlay, B.J., Gibert, J., Hyde, K.D., Johnson, R.K., Kairesalo, T., Lake, P.S., Lovell, C.R., Naiman, R.J., Ricci, C., Sabater, F.F., and Strayer, D.L. 1997. Biodiversity and ecosystem processes in freshwater sediments. Ambio, **26**: 571–577.

Porter, K.G., and Feig, Y.S. 1980. The use of DAPI for identifying and counting aquatic microflora. Limnol. Oceanogr. **25**: 943–948.

Rodriguez, G.G., Phipps, D., Ishiguro, K., and Ridgway, H.F. 1992. Use of a fluorescent redox probe for direct visualization of actively respiring bacteria. Appl. Environ. Microbiol. **58**: 1801–1808.

Rodriguez, P., Martinez-Madrid, M., Navarro, E., and Arrate, J.A. 2000. Selective feeding by *Tubifex tubifex* (Oligochaeta: Annelida). *In* VIII International Symposium on Aquatic Oligochaeta, 18–22 July, Bilbao. University of the Basque Country, Spain.

Sauter, G., and Güde, H. 1996. Influence of grain size on the distribution of tubificid oligochaete species. Hydrobiologia, **334**: 97–101.

Sokal, R.R., and Rohlf, J. 1969. Biometry: the principles and practice of statistics in biological research. W.H. Freeman and Co., Kent, England.

Torreiter, P., Pitaksintorn-Watanamahart, P., and Danielopol, D.L. 1994. The activity of oligochaetes in relation to their ecological role in slow filtration columns. *In* Second International Conference on Ground Water Ecology, 27–30 March, Atlanta. U.S. EPA, AWRA, Herndon, Va. pp. 85–94.

Triska, F.J., Duff, J.H., and Avanzino, R.J. 1993. The role of water exchange between a stream channel and its hyporheic zone in nitrogen cycling at the terrestrial–aquatic interface. Hydrobiologia, **251**: 167–184.

van de Bund, W.J., Goedkoop, W., and Johnson, R.K. 1994. Effects of deposit-feeder activity on bacterial production and abundance in profundal lake sediment. J. N. Am. Benthol. Soc. **13**: 532–539.

Walker, B.H. 1992. Biodiversity and ecological redundancy. Conserv. Biol. **6**: 18–23.

Wavre, M., and Brinkhurst, R.O. 1971. Interaction between some tubificid oligochaetes and bacteria found in the sediments of Toronto Harbour, Ontario. J. Fish. Res. Board Can. **28**: 335–341.

Is catch-per-unit-effort proportional to abundance?

Shelton J. Harley, Ransom A. Myers, and Alistair Dunn

Abstract: We compiled 297 series of catch-per-unit-effort (CPUE) and independent abundance data (as estimated by research trawl surveys) and used observation error and random effects models to test the hypothesis that CPUE is proportional to true abundance. We used a power curve, for which we were interested in the shape parameter (β). There was little difference among species, ages, or gear types in the distributions of the raw estimates of β for each CPUE series. We examined three groups: cod, flatfish, and gadiformes, finding strong evidence that CPUE was most likely to remain high while abundance declines (i.e., hyperstability, where $\beta < 1$). The range in the mean of the random effects distribution for β was quite small, 0.64–0.75. Cod showed the least hyperstability, but still, 76% of the mass of the random effects distribution was below 1. Based on simulations, our estimates of β are positively biased by approximately 10%; this should be considered in the application of our findings here. We also considered the precision of CPUE indices through a meta-analysis of observation error variances. The most precise indices were those from flatfish (median coefficient of variation of ≈0.42).

Résumé : La compilation de 297 séries de données de capture par unité d'effort (CPUE) et des données indépendantes d'abondance (estimées par des inventaires scientifiques au chalut) ainsi que l'utilisation de modèles d'erreur d'observation et d'effets aléatoires nous ont permis de vérifier l'hypothèse que la CPUE est proportionnelle à l'abondance réelle. Nous avons utilisé une courbe exponentielle, dont nous étions intéressés au paramètre de forme (β). Il y a peu de différence entre les espèces, les âges ou les engins de pêche en ce qui concerne la distribution des estimations brutes de β pour chaque série de CPUE. Chez les trois groupes examinés, les morues, les poissons plats et les gadiformes, il y a de fortes indications que la CPUE a une grande probabilité de rester élevée alors que l'abondance décline (i.e., une hyperstabilité, lorsque $\beta < 1$). L'étendue de la moyenne de la distribution des effets aléatoires pour β est très faible, 0,64–0,75. Ce sont les morues qui montrent le moins d'hyperstabilité; néanmoins, 76% de la masse de la distribution des effets aléatoires est inférieure à 1. D'après nos simulations, les estimations de β sont surestimées d'environ 10%, ce qui doit pris en compte dans l'application de nos résultats. Nous avons aussi examiné la précision des indices de CPUE à l'aide d'une méta-analyse des variances des erreurs d'observation. Les indices les plus précis sont ceux des poissons plats (coefficient médian de variation de ≈0,42).

[Traduit par la Rédaction]

Introduction

Despite its well-documented shortcomings, the catch-per-unit-effort (CPUE) from many commercial and recreational fisheries is used in the assessment of fish populations, with strict proportionality between CPUE and abundance frequently assumed. It has long been recognized that CPUE may not accurately reflect changes in abundance (Beverton and Holt 1957).

Paloheimo and Dickie (1964) reviewed the early use of CPUE data in fisheries and argued the importance of understanding both the spatial distribution of fish and the allocation of fishing effort to interpret CPUE data. Possibly the first use of CPUE data in a fisheries setting is attributed to Baranov (1918) who incorporated catch data in the analysis of herring (Seber 1982). Reasons why CPUE might not be proportional to abundance have been investigated by simulation (Sampson 1990; Swain and Sinclair 1994; Gillis and Peterman 1998) and through examination of empirical data (Peterman and Steer 1981; Crecco and Overholtz 1990; Rose and Leggett 1991). The most common form of nonproportionality involves CPUE remaining high while abundance declines. This is known as "hyperstability" (Hilborn and Walters 1992) and can lead to overestimation of biomass and underestimation of fishing mortality (Crecco and Overholtz 1990).

The model of proportionality between CPUE U and abundance N at time t is

$$U_t = qN_t \qquad (1)$$

where q is the catchability coefficient. A number of nonlinear models have been proposed, the simplest being the power curve

$$U_t = qN_t^{\beta} \qquad (2)$$

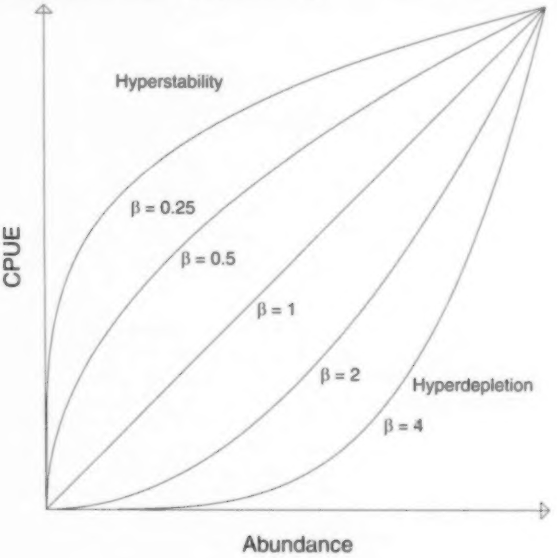
Received December 8, 2000. Accepted June 13, 2001. Published on the NRC Research Press Web site at http://cjfas.nrc.ca on August 16, 2001.
J16127

S.J. Harley[1] **and R.A Myers.** Department of Biology, Dalhousie University, Halifax, NS B3H 4J1, Canada.
A. Dunn. National Institute of Water and Atmospheric Research, P.O. Box 14-901, Kilbirnie, Wellington, New Zealand.

[1] Corresponding author (e-mail: harley@mathstat.dal.ca).

DOI: 10.1139/cjfas-58-9-1760

Fig. 1. Relationship between CPUE and abundance based on different values of the shape parameter β.



where if $\beta = 1$ the model reduces to eq. 1 and if $\beta \neq 1$, then catchability changes with abundance (Fig. 1). When $\beta > 1$, U declines faster than N in a situation known as "hyperdepletion"; conversely, if $\beta < 1$, U declines slower than N, which results in hyperstability. This model does have the property that when $\beta \neq 1$, catchability is no longer given by q, but rather a function of q and β (catchability = $qN^{\beta-1}$). Other nonlinear models have been proposed; Bannerot and Austin (1983) allowed nonlinearity in both effort and abundance, while Richards and Schnute (1986) proposed a very general and flexible range of three-parameter models.

Our interest here is in the relationship between CPUE and abundance and we consider the traditional catchability parameter, q, as a nuisance parameter. We consider the shape parameter of the power curve, β. We prefer to use the power curve, as it requires the estimation of only one additional parameter. In doing so, we admit that we are limiting the forms of the relationship.

While many studies have focused on CPUE for a single stock or fish community, there have been few attempts to compile data across fisheries and examine the problem in a broader context. This type of synthesis or "meta-analysis" has proved useful in many fisheries contexts. A meta-analytical approach has provided insights into the maximum reproductive rate of fish (Myers et al. 1999).

Dunn et al. (2000) examined the relationship between CPUE and abundance for all available New Zealand data sets as well as a number from Atlantic Canada. In general, it was found that the small numbers of paired CPUE/survey observations lead to considerable difficulties in estimating β. They also compared CPUE indices with model estimates of abundance (that had not used CPUE data), as this led to longer time series. Simulations showed that with few paired observations (four to eight), a very large (>50%) reduction in biomass was required to accurately estimate β. When $\beta = 1$, a reduction of up to 75% was required to distinguish between proportional, hyperstable, and hyperdepleted relationships. When the reduction in abundance was low (12.5–25%), $\hat{\beta}$ tended to 1, regardless of the true β.

Here, we compile a large number of CPUE and survey abundance indices from International Council for the Exploration of the Sea (ICES) stock assessment reports. We use commonly accepted observation error models to obtain individual estimates of β and the estimation error variance, which we then combine using basic meta-analytical techniques for the combination of treatment effects.

Methods

CPUE and abundance data

For this study, we require estimates of fishery CPUE and fishery-independent estimates of true abundance. We use research survey abundance estimates as the fishery-independent estimate of abundance. It could also be possible to use biomass estimates from a stock assessment model that did not use CPUE data, but we have not considered this potential data source. With the procedures used here (described in sections below), we require a CPUE time series for a population where there is also available either (*i*) one research survey time series with a reliable estimate of its sampling error, (*ii*) multiple research surveys, or (*iii*) a single "reliable" survey series and multiple CPUE series. We restricted ourselves to data of the second and third types, as this did not require assumptions about the observation error of the survey series.

ICES stock assessment reports offer a standard format and data from a number of fisheries. The larger number of trawl surveys conducted in the ICES region and the length of these series made it possible to obtain a large number of data sets for a range of species (Table 1). The CPUE and survey indices were obtained from the data input files for the stock assessments conducted in 1999. In each case, the CPUE indices were chosen by the experts in each ICES assessment committee as a series representative of the stock status. In some cases the CPUE indices were corrected for fishing power, e.g., horsepower, and sometimes the assessment biologist did not believe that it was necessary. The ICES CPUE indices represent data that are typical of those used by experienced assessment biologists. Details are given in each of the assessment reports available from ICES. The data files can be obtained directly from ICES (http://www.ices.dk/committe/acfm/acfm.htm) or from the corresponding author.

ICES data are usually presented in an age-disaggregated form leading to the large number of data sets available. One limitation of using published data is that in many cases, data were not presented for all ages, e.g., survey data for ages 1–6 and CPUE data for ages 4–9. In such cases, we were only able to use the ages where there was overlap. Also, there were instances where there was no overlap, when survey data were used for the younger ages and CPUE for the older ages. Thus, to a certain extent, we were limited by which data were readily available. We restricted ourselves to data sets where there were at least nine paired observations of CPUE and survey abundance because shorter series are unlikely to produce reliable estimates (Myers and Cadigan 1993; Dunn et al. 2000). This did not result in the exclusion of many data sets.

The CPUE series represented a range of different gear types or fleets (Table 2). For the majority of the trawl data, the specific trawl type was not given. Most gears involve a single vessel towing a net of some type. The Danish seine gear is the only gear that is not towed, but rather, it is set and then hauled, and the pair trawl gear represents a net jointly towed by two vessels.

Table 1. CPUE data used in the meta-analysis.

Species/stock	CPUE series	Ages	Years	Multiple RV surveys
Blue whiting (*Micromesistius poutassou*)				
Northeast Atlantic	1	1–5	12	Yes
Atlantic cod (*Gadus morhua*)				
ICES IV and IIIa (North Sea and Skaggerak)	5	1–5	16	Yes
ICES VIIe–k (Western Channel)	3	1–5	10	No
ICES (Kattegat)	3	2–5	12	No
Four spot megrim (*Lepidorhombus boscii*)				
ICES VIIIc and IXa (West Iberian Shelf)	2	1–7	11	No
Haddock (*Melanogrammus aeglefinus*)				
ICES IV (North Sea)	2	0–5	10	Yes
ICES VIb (Rockall Bank)	3	2–7	10	No
Hake (*Merluccius merluccius*)				
ICES IIIa, IV, VI, VII, and VIIIab (North)	4	2–5	12	No
Megrim (*Lepidorhombus whiffiagonis*)				
ICES VII and VIIIab (Bay of Biscay)	1	2–9	9	Yes
ICES VIIIc and IXa (West Iberian Shelf)	2	1–7	10	No
Plaice (*Pleuronectes platessa*)				
ICES VIIe (Western Channel)	3	3–5	10	No
ICES VIIfg (Celtic Sea)	3	2–5	9	No
ICES VIIa (Irish Sea)	2	2–4	10	No
ICES VIId (English Channel)	3	2–5	9–10	Yes
Pollock (*Pollachius virens*)				
ICES IV and IIIa (North Sea and Skaggerak)	3	3–7	9	No
Sole (*Solea vulgaris*)				
ICES VIIa (Irish Sea)	2	3–9	10–11	No
ICES VIId (English Channel)	2	2–4	11	No
ICES IV (North Sea)	2	2–4	13	Yes
ICES VIIfg (Celtic Sea)	2	2–5	11	No
ICES VIIIab (Bay of Biscay)	2	1–8	11	No
ICES VIIe (Western Channel)	2	3–6	15	No
Whiting (*Merlangius merlangus*)				
ICES IV (North Sea)	3	2–5	16	Yes
ICES VIa (West of Scotland)	3	2–6	11	No
ICES VIIe–k (Celtic Sea)	2	1–6	12	No

Note: RV, research vessel.

Observation error models

PROC CALIS (Covariance Analysis and LInear Structural equations) estimates parameters of linear structural equations using covariance structure analysis (SAS Institute Inc. 1990). This is a general framework that can include observation error models as a special case. To obtain individual population estimates of β, we use the power curve

$$U_t = qN_t^{\beta}e^{\epsilon_t}$$

Model notation is summarized in Table 3.

We take the logarithm of the different components such that $n_t = \log(N_t)$ and $u_t = \log(U_t)$. An important assumption that we will use is normality of the independent variable, so we have $n_t \sim \mathrm{N}(\mu,\phi)$, which states that the true abundances, N_t, are independently lognormally distributed.

As we do not actually observe the true abundances, rather estimates from research trawl surveys, the observation error in n_t can be incorporated into the model by

$$o_t = n_t + \zeta_t$$

$$o_t \sim \mathrm{N}(\mu, \phi + \sigma_{\zeta}^2)$$

where o_t is the observed value of n_t from the survey and the observation error is assumed to be normal, i.e., $\zeta_t \sim \mathrm{N}(0,\sigma_{\zeta}^2)$. For CPUE, we can write

$$u_t = \log(q) + \beta n_t + \epsilon_t$$

$$u_t \sim \mathrm{N}(\log(q) + \beta\mu, \beta^2\phi + \sigma^2)$$

where β is the slope of the relationship between the logarithm of CPUE and the logarithm of the abundance and σ^2 is the estimation error variance. From these assumptions, the theoretical variance–covariance matrix (Σ) of the joint distribution of the CPUE series (u_t) and the observed survey series (o_t) is

$$(3) \qquad \Sigma = \begin{bmatrix} \phi + \sigma_{\zeta}^2 & \beta\phi \\ & \beta^2\phi + \sigma^2 \end{bmatrix}$$

where the parameters to estimate are β, ϕ, σ_{ζ}^2, and σ^2. This problem is not identified, i.e., unique solutions do not exist, unless one of the four parameters can be estimated independently. This is because there are four unknown parameters but only three elements of Σ. This example represents a standard structural regression problem.

The identification problem is overcome if we have multiple surveys or multiple CPUE series. If we have two surveys, and with the same assumptions as before, the survey estimates are

$$o_{t,1} = n_t + \zeta_{t,1}, \text{ where } \zeta_{t,1} \sim \mathrm{N}(0, \sigma^2_{\zeta_1})$$

$$o_{t,2} = n_t + \zeta_{t,2}, \text{ where } \zeta_{t,2} \sim \mathrm{N}(0, \sigma^2_{\zeta_2})$$

and the theoretical variance–covariance matrix of the CPUE and two survey series is

$$\Sigma = \begin{bmatrix} \phi + \sigma^2_{\zeta_1} & \phi & \beta\phi \\ & \phi + \sigma^2_{\zeta_2} & \beta\phi \\ & & \beta^2\phi + \sigma^2 \end{bmatrix}$$

where the parameters to be estimated are $\sigma^2_{\zeta_1}, \sigma^2_{\zeta_2}, \sigma^2, \beta$, and ϕ. As there are more free parameters than unique elements of the observed variance–covariance matrix, the model is identified. It is simple to extend Σ for the case of more than two surveys and the case of multiple CPUE series and a single survey series. The case of two CPUE series and one survey series is exactly identified. We used all trawl surveys that were available (and used in the stock assessment) for a stock (two to four) (Table 1).

We used maximum likelihood methods to estimate the parameters of Σ (details of the methods can be found in Bollen (1989)). The basic estimation procedure is to fit the observed variance–covariance matrix with the theoretical one. The fitting function that is minimized to find the maximum likelihood estimates is

$$(4) \qquad \log|\Sigma| + \mathrm{tr}(\mathbf{S}\Sigma^{-1}) - \log(|\mathbf{S}|) - d$$

where "tr" is the matrix trace and d is the dimension of **S** (Bollen 1989). Maximum likelihood does not always produce sensible results, i.e., the parameters describing the variance can be estimated to be <0 if they are not constrained. This often happens with short time series in which the sample variance is not an adequate approximation to the true variance. Any model in which at least one of the estimates of the variance parameters had to be constrained to be >0 or whose estimates were very small (<0.06) was rejected. We will return to the robustness of and alternative approaches to this problem later. Standard errors are estimated using the inverse of the Hessian matrix.

We use a structural as opposed to a functional regression approach for estimation in the presence of measurement errors (Fuller 1987). In the structural approach, we make the assumption that the independent variable, i.e., the true abundance, is a random variable that has a common distribution. The functional approach, which we did not use, assumes that the independent variables are fixed, and each must be estimated. The functional approach has the advantage that changes over time can be easily modeled but has the considerable disadvantage that a separate unknown abundance would have to be estimated for each year. This leads to an estimator that is not guaranteed to be consistent because more parameters are estimated as more data are added (Fuller 1987).

Meta-analysis of individual parameter estimates

A range of techniques exist for combining effect sizes or a parameter of interest across studies or populations (Cooper and Hedges 1994). Here, we will combine estimates of β and the estimation error variance (σ^2) for each data set within a Gaussian "random effects" framework. We assume that there is an underlying distribution for the parameter of interest. If we consider the shape parameter β, we assume that each of the true unobserved β_is are normally distributed, i.e., $\beta_i \sim \mathrm{N}(\mu_\beta, \sigma^2_\beta)$. We also assume that each of the true values for β_i is estimated with error. We assume normal observation error, $\hat{\beta}_i \sim \mathrm{N}(\beta_i, v_i)$, where v_i is the estimation variance, $(\widehat{\mathrm{SE}(\beta_i)})^2$. Finally, the variability observed in $\hat{\beta}_i$ (i.e., all of the individual estimates) can be written as a sum of two variances:

$$\mathrm{var}(\hat{\beta}_i) = \sigma^2_\beta + v_i$$

We followed the maximum likelihood approach of Hardy and Thompson (1996) for combining the estimates of β_i. This approach allows the estimation of both the underlying distribution from which the β_is are drawn and the profile log likelihood, which can be used to calculate confidence limits. The form of the joint log likelihood in terms of μ_β and σ^2_β is

$$(5) \qquad l(\mu_\beta, \sigma^2_\beta) = -\sum_{i=1}^{k} \frac{1}{2} \log 2\pi(\sigma^2_\beta + v_i) - \sum_{i=1}^{k} \frac{(\hat{\beta}_i - \mu_\beta)^2}{2(\sigma^2_\beta + v_i)}$$

We repeated the analysis for the estimation error variance of the relationship between CPUE and abundance (σ^2_i). To meet the assumption of normality, we assumed that $\log \sigma_i$ was a normal random variable (i.e., $\log \sigma_i \sim \mathrm{N}(\mu_{\log \sigma}, \sigma^2_{\log \sigma})$). We also assumed that the errors were normal so that $\widehat{\log \sigma_i} \sim \mathrm{N}(\log \sigma_i, \omega_i)$. We derived approximate estimates of ω_i using the delta method. An advantage of using $\log \sigma$ is that σ is approximately equal to the coefficient of variation (CV) of the series under reasonable conditions. For this reason, we will discuss our results in terms of σ.

The random effects distribution could also be seen as a "prior" distribution that we would expect individual values of a parameter to be from. As we used maximum likelihood estimation (MLE), the random effects distribution can be referred to as the MLE prior (Efron 1996), i.e., the maximum likelihood estimate of the prior distribution. In this case the MLE prior distribution reflects the distribution from which the true individual values of a parameter are drawn. We prefer this term here, as it better reflects how these distributions might be used in practice.

Results

Large fluctuations in the CPUE and survey indices were observed. The median relative range (maximum/minimum CPUE index) in CPUE was approximately 12-fold, and the 95th percentile was 0.69. This suggests that there is enough contrast in the data to estimate β. It was also found that the lowest point in a CPUE series was just as likely to be the first or last year, although overall the median decline in log CPUE was 5% per year.

While it is not possible to display all data used in the analysis, nine randomly selected data sets from the 297 considered in the analysis (using a random number table) are presented in Fig. 2. Seven of the nine series had estimates of $\beta < 1$ from our maximum likelihood structural models. The series in Fig. 2*d* exhibited hyperdepletion, and the series in Fig. 2*g* did not provide feasible results (i.e., negative variance estimates described below). From an examination of the data, there is no evidence for more complex models.

Of the 297 data sets that were analyzed, 209 were retained for the subsequent meta-analysis. That is, data sets were excluded if the maximum likelihood estimates for σ^2 were negative or if the estimates for ϕ were either negative or very small (<0.06). In the case where ϕ was very small, there was very little estimated variation in true abundance, and thus, it was not possible to obtain accurate estimates of how CPUE

Table 2. Gear types of CPUE series.

Code	Gear type	Data sets
BeTrawl	Beam trawl	55
DSeine	Danish seine	32
LTrawl	Light trawl	32
NTrawl	Shrimp trawls	20
OTrawl	Otter trawl	12
PTrawl	Pair trawl	5
Trawl	Unspecified trawl	142

Table 3. Summary of notation used in this paper.

Symbol	Description
U_t	CPUE index in year t
q	Catchability coefficient
N_t	Abundance in year t
β_i	Shape parameter of the power curve for CPUE series i
ϵ_t	True log deviation from the power model in year t
o_t	log of true abundance (unobserved) in year t
σ_ζ^2	Observation error variance of the log-transformed survey
ϕ	Variance in true abundance of the log-transformed time series
μ	Mean true abundance of the log-transformed time series
σ^2	Estimation error variance of the log-transformed CPUE series
$\log \sigma$	log of the square root of the estimation error variance of the CPUE series
ν_i	Estimation variance for β_i, $(\widehat{\mathrm{SE}(\beta_i)})^2$
ω_i	Estimation variance for $\log \sigma_i$, $(\mathrm{SE}(\widehat{\log \sigma_i}))^2$
Σ	Theoretical variance–covariance matrix
$\mathbf{S}$	Observed variance–covariance matrix
μ_β	Mean of random effects distribution for β
σ_β^2	Variance of random effects distribution for β
$\mu_{\log \sigma}$	Mean of random effects distribution for $\log \sigma$
$\sigma_{\log \sigma}^2$	Variance of random effects distribution for $\log \sigma$

changed with abundance (i.e., regression with estimates of the dependent variable at only a single value of the independent variable). Sensitivity to this cutoff criterion is discussed at the end of the results section and was investigated by the simulations of Myers and Cadigan (1993).

We compared the data series where estimates of β were obtained and those that were excluded. No differences were found in the length of series or contrast in the CPUE or survey indices. It was found, however, that the presence of multiple surveys was different between the two groups. For data sets where multiple surveys were available, 87% of the series gave acceptable results (positive variances) versus 67% for those without. This finding was not surprising, and we then compared the results of those data sets that gave acceptable results on the basis of whether there were multiple surveys or not. There was evidence (not statistically significant by an analysis of medians) that the individual estimates of β were greater for those series without multiple surveys (median of 0.84 versus 0.67). However, the standard errors were also higher, indicating that these estimates were less precise. Overall, the variance estimates were lower and less variable for the series with multiple surveys.

Except perhaps for data from the one hake stock, the distribution of the shape parameter β did not differ significantly across the species (Fig. 3), and most of the $\hat{\beta}_i$s were <1. Estimates for hake were mostly >1 and considerably different from those for other species that were examined. Possible problems with these data will be discussed later. For species other than hake, in most cases the upper quartile of the distribution of β was <1 and the upper 95% confidence limit for the median was <1. Data for the youngest (0) and oldest ages (8 and 9) were variable, but there were few observations for these ages. There is some evidence that the upper quartile increases with age, but any trend in median $\hat{\beta}$ at age is unclear (Fig. 3). None of the gear types appear to be significantly different from one another. Aside from the median $\hat{\beta}$ for the otter trawl, which is close to 1, the median for all of the other gear types is approximately the same ($\approx$0.7). The 95% confidence limits for the medians for both beam trawl and trawl are below 1. Only one obvious outlier was present among the estimates of β_i; this was an estimate of –3.23 for age-1 whiting in ICES area VIIek for a nephrops trawl. This estimate had a high standard error (2.31) and had very little influence on the subsequent random effects meta-analysis.

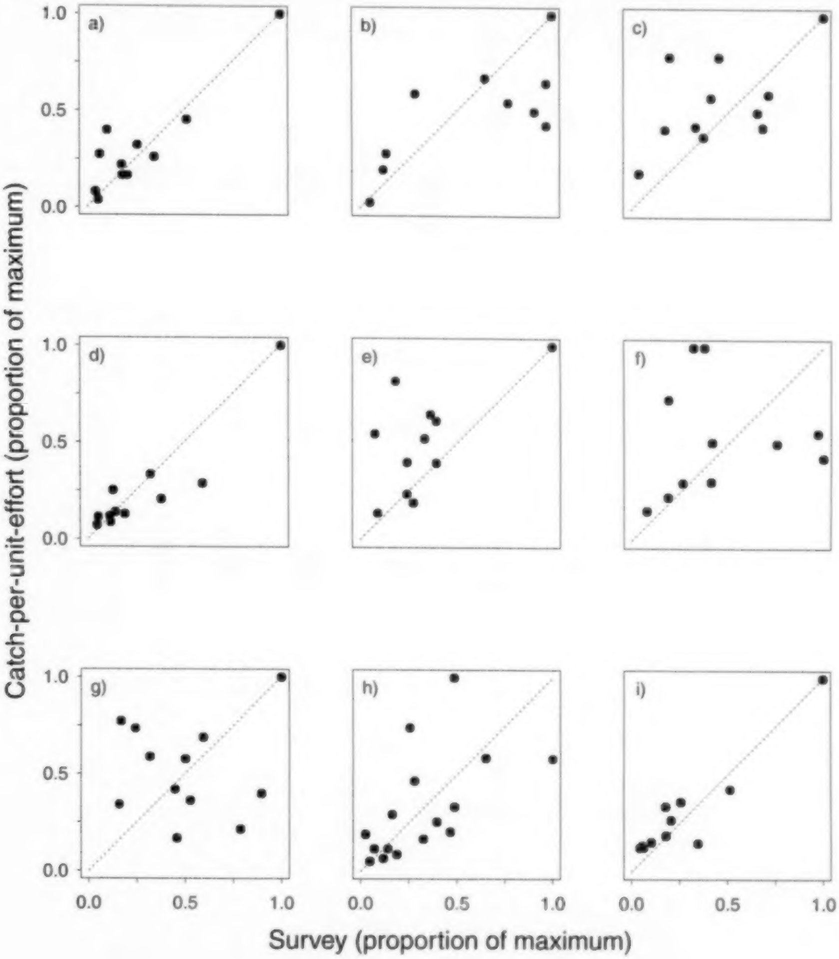
Sole and whiting had the lowest median $\hat{\sigma}$ ($\approx$0.4) (Fig. 4). Estimates for the flatfish species were generally lower (but not significantly different) than those of the gadiformes. The highest estimates of σ were for haddock, with the upper quartile >0 (i.e., CV > 1). The youngest ages had the highest estimates of σ, and the distributions of σ were very similar for the other ages. Beam trawl estimates of σ showed little variation and had the lowest median (but not significant different) of the gear types. Trawl estimates were also tightly distributed.

Estimates of the MLE priors for both β and $\log \sigma$ for cod were the least certain, as indicated by the wide 95% confidence regions (Fig. 5). The random effects variances were also largest for cod, indicating higher inherent variability in the true distributions. However, the sample size for cod was less than for the other groups. The confidence region for the gadiformes was within the cod regions. The variance of the MLE prior for $\log \sigma$ for flatfish was 0. For none of the groups does the 95% confidence joint region include $\hat{\beta} > 1$.

For all groups, most of the $\hat{\beta}_i$ s were between 0.6 and 0.9 (Fig. 6). The mean of the MLE prior for both cod and gadiformes was similar (0.75 and 0.73, respectively) and higher than that for flatfish (0.64). The variances of the MLE priors were similar for all groups. The mean of the MLE prior for $\log \sigma$ was the same for both cod and the gadiformes (–0.65), which represents a median CV of 0.52 (Fig. 7). This was lower than for flatfish (–0.86, representing a CV of 0.42). This suggests that flatfish CPUE provides more precise abundance indices (i.e., lower noise) than those of gadiformes.

We repeated the random effects analysis for β using different criteria for inadmissible estimates of variances. The original criteria was $\phi < 0.06$, so cutoffs of 0 and 0.1 were examined for sensitivity. None of the estimates of μ_β or σ_β^2 varied by more than 5% from the 0.06 case used for the

Fig. 2. Nine randomly chosen series of CPUE and an associated series of trawl survey abundance. Both series are scaled to a maximum of 1. Estimates of $\beta < 1$, implying hyperstability, are associated with data sets, with the majority of points above the 1:1 line (dashed) that is provided for reference. (*a*) Age-4 sole, Irish Sea, trawl, $\hat{\beta} = 0.73$; (*b*) age-3 megrim, ICES VIIIc and IX, trawl, $\hat{\beta} = 0.87$; (*c*) age-4 whiting, ICES VIa, Danish seine, $\hat{\beta} = 0.38$; (*d*) age-6 sole, Irish Sea, beam trawl, $\hat{\beta} = 1.20$; (*e*) age-5 sole, ICES VIId, beam trawl, $\hat{\beta} = 0.66$; (*f*) age-3 whiting, ICES VIa, Danish seine, $\hat{\beta} = 0.80$; (*g*) age-4 blue whiting, Northeast Atlantic, pair trawl, $\hat{\beta} = -21.70$; (*h*) age-3 plaice, ICES VIId, beam trawl, $\hat{\beta} = 0.53$; (*i*) age-4 sole, Irish Sea, beam trawl, $\hat{\beta} = 0.62$.



analysis. Similar results were obtained for different cutoffs of the estimation error variances.

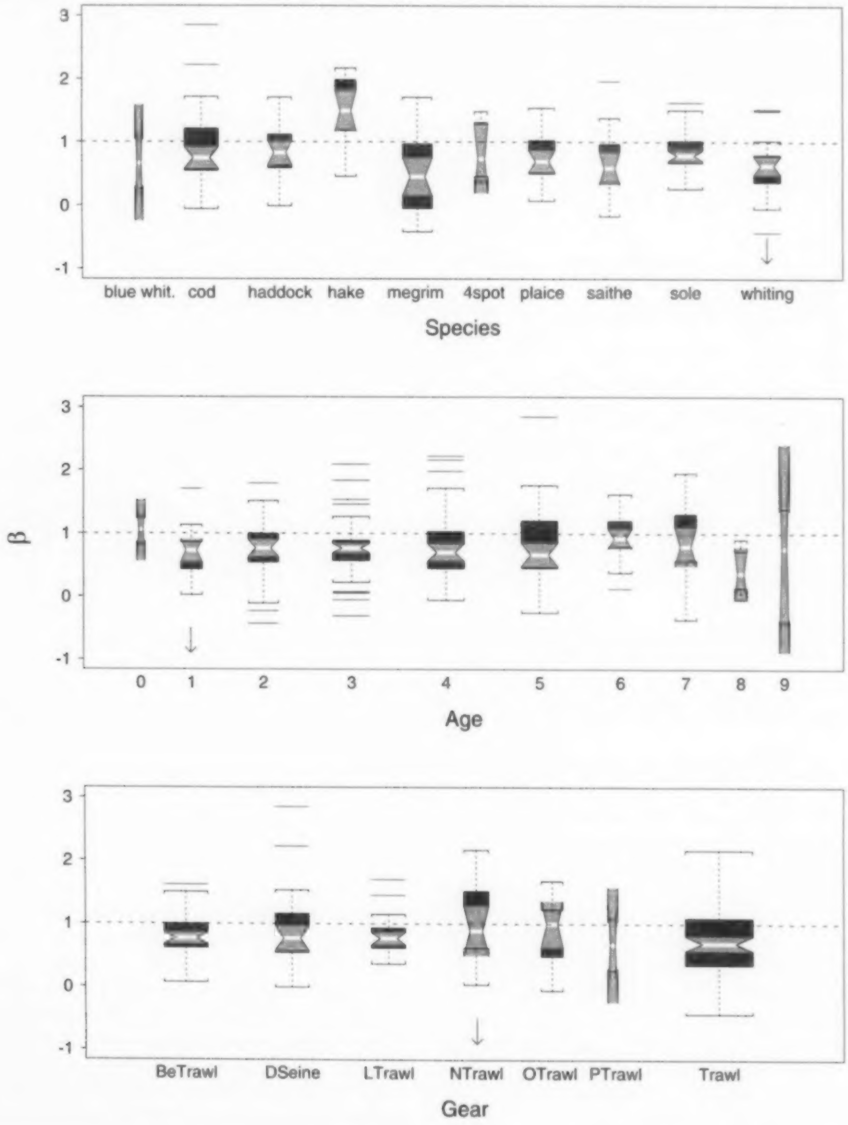
Discussion

While the validity of CPUE as proportional index of abundance has long been questioned, our analysis provides the first large-scale attempt to test the assumption of proportionality. Our findings here, while based on data from a single area, must lead to concern among those who model CPUE data assuming proportionality. Focusing on data from the ICES region, we obtained 297 data sets of which we were able to obtain estimates of β for 209. We have taken data from ICES assessments because it was possible to get large amounts of data in a standard format.

It was found that the availability of multiple survey series was related to the ability to obtain reasonable estimates of β. Of the series where estimates of β were possible, only 62 (30%) were >1. It is not surprising that most of the estimates were <1; in fact, many researchers have found evidence of hyperstability in the relationship between CPUE and abundance (e.g., Peterman and Steer 1981; Crecco and Overholtz 1990; Swain and Sinclair 1994). We believe now that it is time to move on and incorporate this new information into stock assessment models.

The MLE prior for β had most of its weight well below 1 for all three groups. For cod, 76% of the mass was below 1, and this was lower than that for flatfish (86%) and gadiformes (81%). This represents the first large-scale examination of the relationship between CPUE and abundance, and as such provides the strongest evidence against the as-

Fig. 3. Summary of shape parameter β by species, age, and gear type. The boxplots show the limits of the middle half of the data (the line inside the box represents the median). The amount of data is shown as the width of the boxes that are proportional to the square root of the number of data points. The notches are the approximate 95% confidence intervals of the median. If the notches on two boxes do not overlap, this indicates a difference at a rough 5% significance level. The upper quartile and lower quartile provide the outline of the box. Whiskers are drawn to the nearest value not beyond 1.5(interquartile range) from the quartiles; points beyond are drawn individually as outliers. The arrows indicate the outlier with $\hat{\beta} = -3.23$ (details given in text). Details of the species and gear types are given in Tables 1 and 2, respectively.

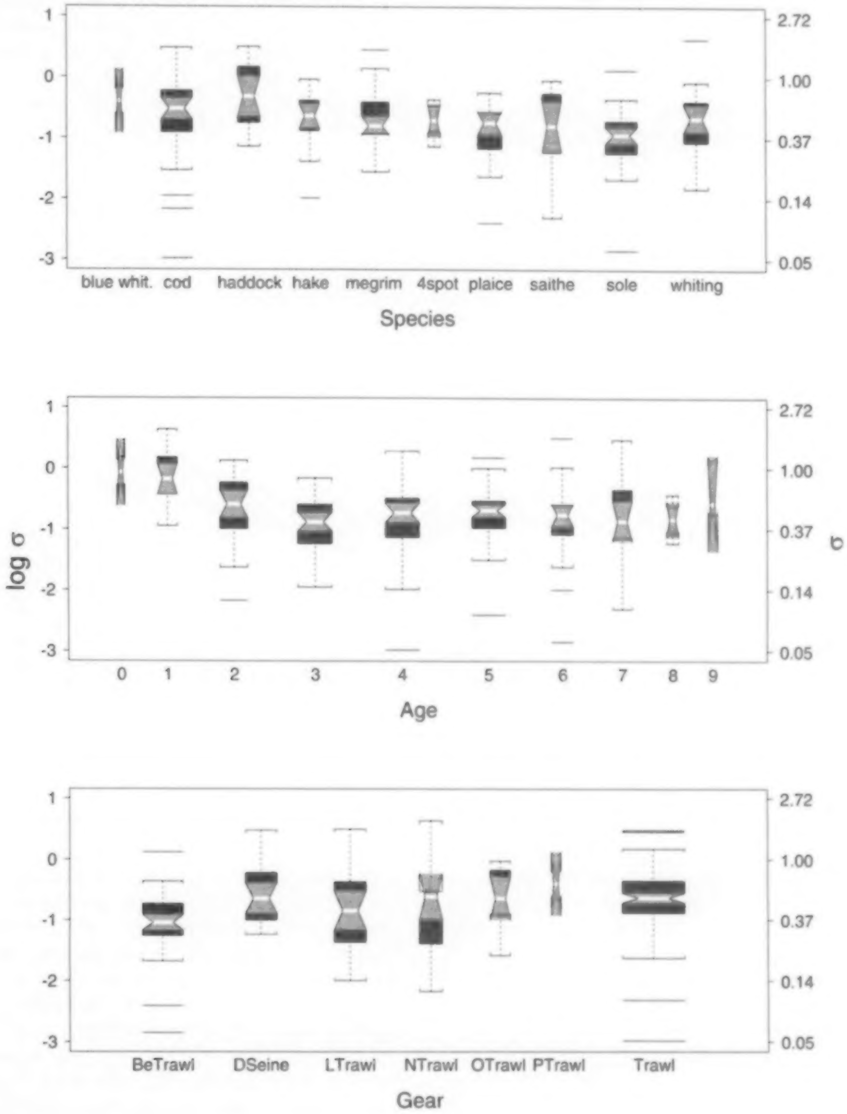


sumption of proportionality. However, here, we have only examined a limited number of gear types, mainly trawl-type gears. For example, we have not examined any CPUE series from purse-seine fleets, although the fishing process of these types of vessels has been studied previously, with evidence of hyperstability (Clark and Mangel 1979; Allen and Punsly 1984).

There do exist stock assessment procedures that consider nonlinearity in the relationship between CPUE and abundance (e.g., Darby and Flatman 1994). However, this assessment package does not currently have the ability to incorporate information on β, such as that provided by the MLE priors presented here. Furthermore, in many ICES assessments the power curve is most commonly used for only the youngest ages-classes. Here, we show that the power curve is the appropriate model at all ages.

Bayesian assessment methods allow the incorporation of additional information in the form of prior distributions

Fig. 4. Summary of the log of the estimation error standard deviation (log σ) by species, age, and gear type. See Fig. 3 for a description of the boxplot.



(Walters and Ludwig 1994; Punt and Hilborn 1997; Meyer and Millar 1999). In Bayesian analysis, the prior distributions are combined with the likelihood components for the data to give posterior probability distributions that are used for inference. It would also be possible to use the MLE priors as penalty factors or as an additional component of the total likelihood in a non-Bayesian analysis.

We have also obtained estimates of the observation error variance of a CPUE series, log σ. As σ represents the square root of the observation error variance of a CPUE series, if the distribution of the ϵ_ts is approximately lognormal, then σ is an approximation of the CV of the CPUE data source. This gives an indication of the precision of CPUE indices and is extremely important for situations where alternative data types (e.g., CPUE and trawl survey) are available for a stock assessment. In such cases the weighting of the different data sources can be important (Quinn and Deriso 1999). This distribution for log σ will also be useful for simulation studies. We found that log σ was generally lower for flatfish than for the gadiformes. This implies that CPUE data from flatfish will be more useful for "tuning" stock assessment models.

Here, we have provided estimates of the MLE prior for β as well as confidence regions based on the log likelihood profiles. The MLE prior represents the distribution from which we can expect the true values of β_i to be drawn. The variance of this distribution may be an underestimate of the true variance, as it does not consider the uncertainty in the estimation of β and σ_β^2 (Efron 1996). For this reason, it may be important to "increase" the variance by some factor before using these distributions as Bayesian priors or as additional likelihood components.

In some fisheries, research survey abundance indices may not be available or the survey series may be quite recent and not provide information on historical stock sizes. If CPUE data exist, it may be possible to get some indication of historical stock sizes using the priors for β that we have derived here.

Robustness and alternative methods

The analysis presented here is completely reliant on the assumption that research surveys indices are proportional to true abundance. If the surveys were carried out within some random sampling framework, then one would hope that this assumption would hold. However, in some cases the surveys may not cover the whole stock. In such circumstances, proportionality may still hold, but nonproportionality is also possible. By using the data used by the assessment scientists, we are making the same assumptions that they have made. Given this, we are more confident in estimates for stocks where multiple surveys were available or the surveys provided good coverage of the stock area.

The model used to estimate density-dependent catchability was used in a slightly different formulation to estimate density-dependent mortality by Myers and Cadigan (1993). Cadigan (1993) and Myers and Cadigan (1993) carried out extensive simulations to determine the effect of small sample size and violations of the model assumptions on bias of the parameter mathematically equivalent to our β. Both studies found that reasonable violations of the model assumptions (alternative distributions for the error distributions, autocorrelation, and time trends in the series) had relatively little effect on bias but that small sample size generally led to overestimation of the parameter mathematically equivalent to our β parameter by ≈10% and underestimation of the estimation error variance by ≈10%. Cadigan (1993) developed two methods to correct for bias in the individual estimates but found that they did not work well in practice. We recommend that the estimates of β presented here be reduced by 10% to account for the general trends in bias observed in these simulation studies.

We only considered one model for the relationship between CPUE and abundance (eq. 2). This power curve is the simplest model allowing nonlinearity between CPUE and abundance. There was no evidence that more complicated models would provide more parsimonious fits to the data. In fact, even with a large number of data sets, it was difficult to obtain reliable estimates of the shape parameter of the power curve.

We used maximum likelihood to estimate the parameters of the random effects distributions, but other approaches are possible. A moment estimator for the mean of the random effects distribution was provided by DerSimonian and Laird (1986). This approach required that the variance of the random effects distribution (σ_β^2) was known, and thus, no estimate of its uncertainty is possible. The likelihood approach presented by Hardy and Thompson (1996) enables the analyst to obtain profile-based confidence limits for both the mean and the variance of the random effects distribution. It would also be possible to use hierarchical Bayesian methods to estimate the random effects distribution (Carlin and Louis 1996; Liermann and Hilborn 1997). When using Bayesian methods, it is necessary to specify prior distributions for the mean and variance of the random effects distribution. In instances where the maximum likelihood estimates for the variance are significantly different from 0, it is unlikely that the prior specification for variance in a Bayesian model would lead to estimates different from those from the likelihood approach. However, in cases where the data are poor, it is common for the maximum likelihood estimate of random effects variance to be 0 (Searle et al. 1992). In such circumstances, the choice of prior for the Bayesian analysis is likely to be important.

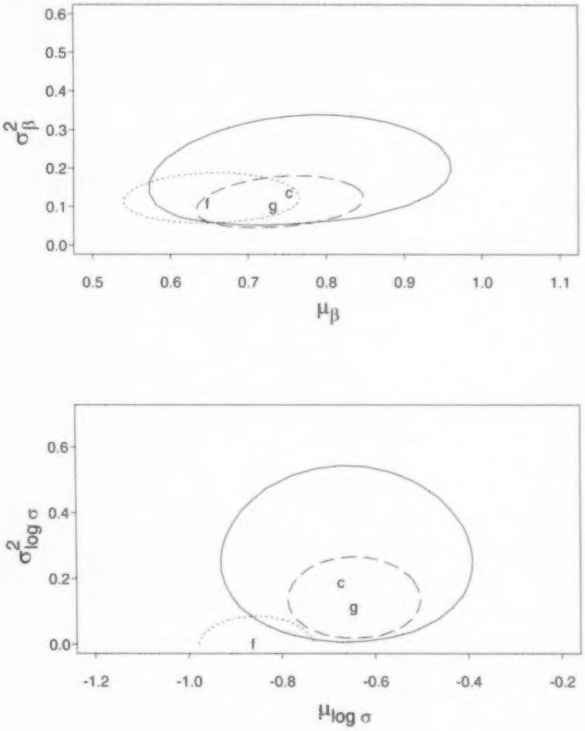
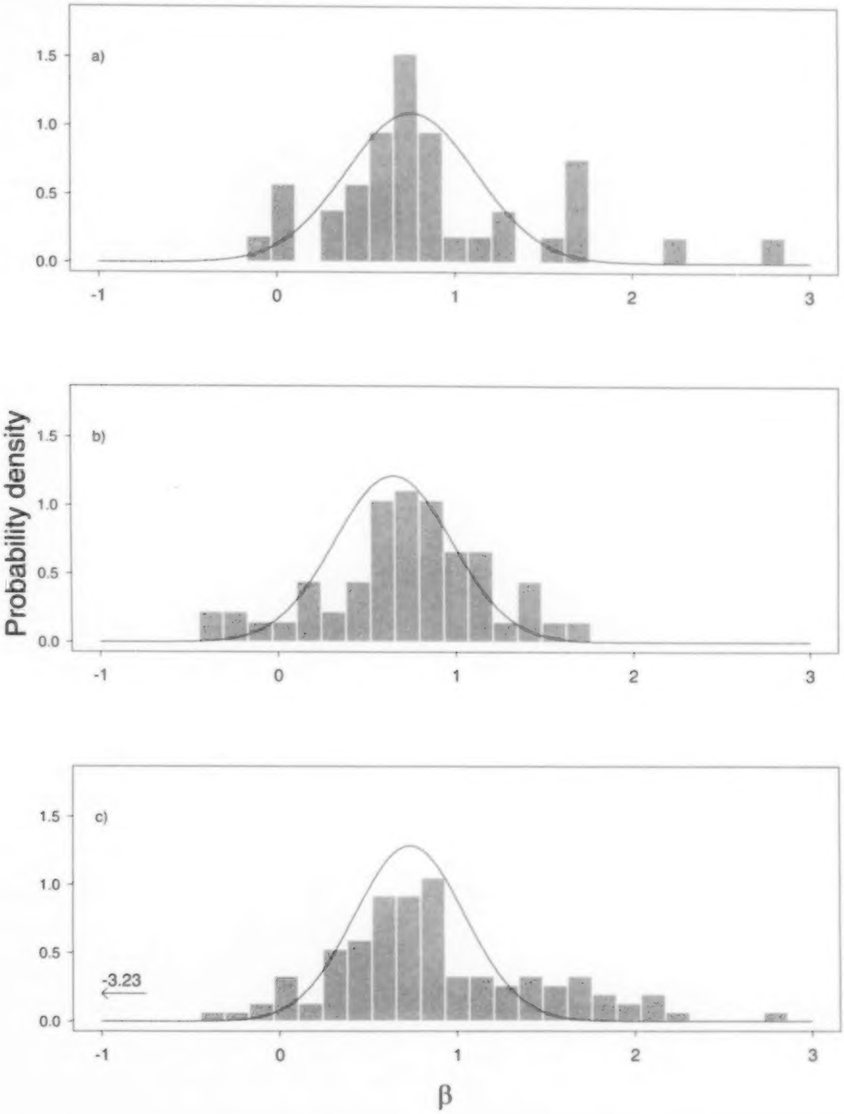


Fig. 5. Log likelihood contours for μ_β and σ_β^2 (top) and $\mu_{\log\sigma}$ and $\sigma^2_{\log\sigma}$ (bottom) for cod (solid line), flatfish (dotted line), and gadiformes (dashed line). The contours represent the approximate 95% joint confidence region ($\chi^2_{0.05,2}$ / 2 ≈ 2.991). The maximum likelihood estimates for each group are indicated by lowercase letters, i.e., c for cod, f for flatfish, and g for gadiformes.

It is also possible to do a fully Bayesian analysis of these data. The individual modeling of the relationship between CPUE and abundance for each series could be performed simultaneously, where the β_is are estimated, while considering the information provided by other data sets. In such a case, we would estimate the prior distribution within the analysis rather than separately as we have done here. This type of approach has been used in the estimation of the maximum reproductive rate from spawner–recruitment data (Myers et al. 1999). While this approach is theoretically possible, it would be computationally intensive but will be the focus of future

Fig. 6. Histogram of individual estimates of β_i with overlaid MLEs of the prior. The histogram has been scaled to sum to 1 so that it is directly comparable with the prior density function. (*a*) Cod: based on 38 data sets; the MLE prior for β was $\beta \sim N(0.75, 0.13)$; (*b*) flatfish: based on 98 data sets, $\beta \sim N(0.65, 0.11)$; (*c*) gadiformes: based on 111 data sets, $\beta \sim N(0.73, 0.10)$. The arrow indicates the outlier described in the text.



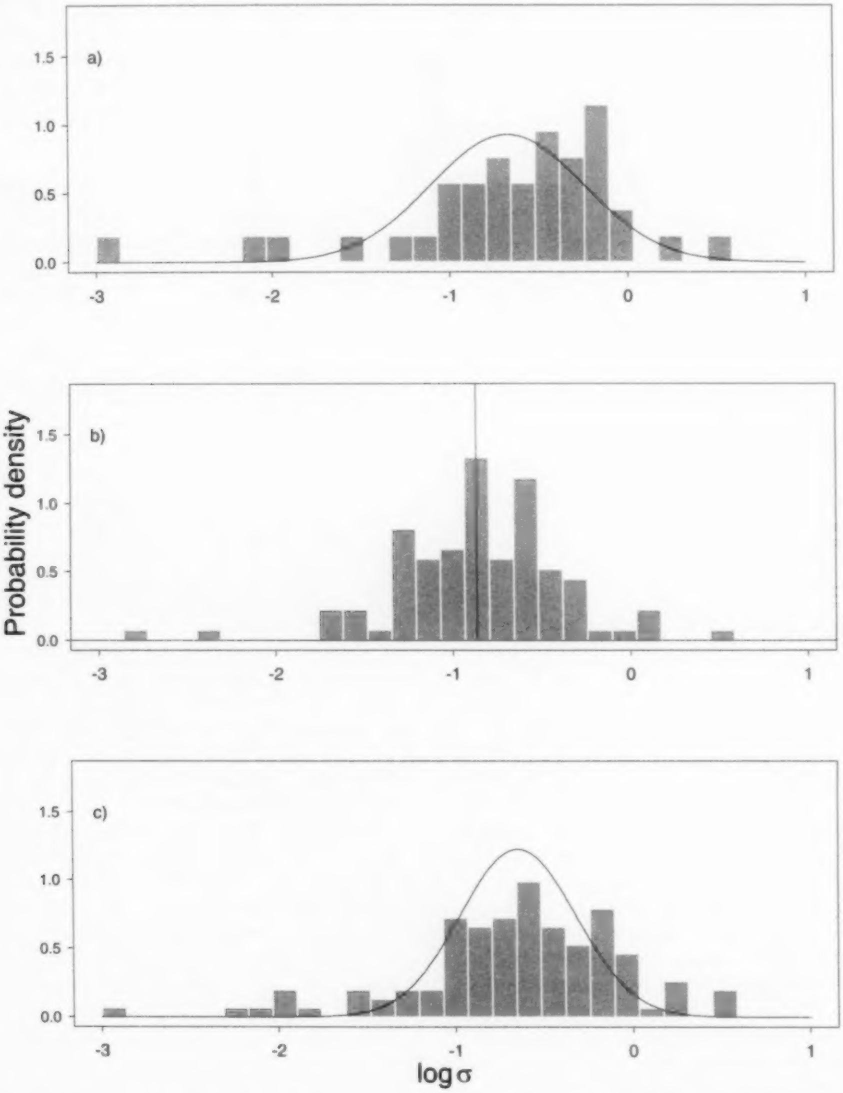
research. It is possible that this approach would help overcome some of the problems encountered estimating the variance components.

The data used for this analysis were series of age-specific CPUE and research survey abundance indices as used in ICES stock assessments in 1999. As ICES assessment scientists often have access to a number of CPUE and survey series, the data used in the assessment models may be of better quality than those used by assessment scientists with less available data (i.e., they have enough data to consider not using less reliable series). Thus, it is possible that the data used in this analysis are better than those used by assessment scientists elsewhere. Data series that exhibited extreme hyperstability or large observation error may have been excluded by the assessment scientists. If the data that we have included are less noisy (regardless of the value of β), our estimates of the MLE prior for log σ will overestimate the usefulness of CPUE as a predictor of abundance (on the whole).

We have assumed that estimates for the different ages are independent (in the random effects analysis). This may cause us to overestimate the effective number of data sets that we have. This would not bias our results but could cause the confidence regions in Fig. 5 to be underestimated.

Dunn et al. (2000) used both research survey (both acous-

Fig. 7. Histogram of individual estimates of the log of the estimation error standard deviation (log σ_i) with overlaid MLEs of the prior. The histogram has been scaled to sum to 1 so that it is directly comparable with the probability density function. (*a*) Cod: based on 38 data sets; the MLE prior for log σ was log σ ~ N(–0.67, 0.18); (*b*) flatfish: based on 98 data sets, log σ ~ N(–0.86, 0.0); (*c*) gadiformes: based on 111 data sets, log σ ~ N(–0.65, 0.11).



tic and trawl) and model-based (e.g., predicted abundance from a statistical catch-at-age model) estimates of abundance in examining the relationship between CPUE and abundance. We have chosen not to use model-based abundance estimates for four reasons, aside from problems that might occur due to biases in the assessment methods. First, there is seldom one single model that is used in any one assessment. A number of structurally different models may be compared and it would require us to choose between models. Second, for the model-based abundance indices to be independent of the CPUE series, we would require that the CPUE data not be included in the model. In many cases, this type of model may not have been considered. Third, it would be difficult to obtain realistic estimates of the observation error variance for model-based estimates. Finally, the model-based abundance indices are unlikely to be independent from one year to the next as an underlying population dynamics model generates them. This would complicate any analysis, as it would require the incorporation of autocorrelation in the estimation procedure. Thus, while model-based abundance indices have the advantage that they synthesize a range of information on abundance, we felt that the disadvantages of model-based indices outweigh this.

We have used age-disaggregated abundance indices as opposed to the aggregated indices used by Dunn et al. (2000). While aggregated indices may be less noisy (due to averaging of error over a number of ages), any differences in the age-specific selection of the survey and fishing gears will lead to biases in the estimation of β. It is also easier to get good contrast with age-structured indices due to variability in recruitment. Any ageing error would most likely act to reduce the interannual variability in abundance of the older age-classes. The median relative range (maximum/minimum CPUE index) in CPUE for a series was 12-fold. Based on the simulations performed by Dunn et al. (2000), the variability observed here is sufficient to allow accurate estimation of β.

We have used age-specific estimates of density-dependent catchability, β, as opposed to the more common practice of estimating a single parameter by combining data across all ages. The relationship between age-aggregated CPUE and abundance is likely to be different, so we do not suggest that our results be applied directly in this situation. The relationship between CPUE and abundance is probably a function of total abundance rather than the abundance of individual cohorts. We carried out simulations where we fixed a single β for the entire population and then estimated a separate β for each age-class. We found that the estimates were always closer to 1, suggesting that our age-based estimates may overestimate the extent to which CPUE is proportional.

Changes in catchability, i.e., efficiency of the fishing fleet over time, have the potential to cause biases in the estimates of β if there are trends over time in abundance. For our CPUE data, the lowest value in a series was just as likely to be the first or last year, but the median time trend was a 5% decline per year. We therefore do not believe that this will have a substantial effect on the mean of our estimates of β.

This investigation leads itself to obvious extensions. A meta-analysis of log σ_ζ (the square root of the estimation error variance from the research surveys) will provide information on the general reliability of research survey data. When combined with the analysis presented here, this will lead to more accurate weighting of alternative data sources.

It is also important to determine the generality of the findings here. It will be important to examine other data, especially from other gear types, to determine if hyperstability is a property of other fishing methods (e.g., longlining and recreational hook and line fisheries).

It will also be important to determine the extent to which β can be predicted for a given CPUE series and population. It is clear that both fish and fisher behavior can lead to hyperstability. Myers and Cadigan (1995) found that northern cod exhibited a substantial contraction in range during the period up to the closure of the fishery, and Hutchings (1996) showed that the areas of high cod density remained constant throughout this time. Density-dependent habitat selection theory (Fretwell and Lucas 1970; MacCall 1990) suggests that range expansion and contraction could occur with changes in abundance. To better understand the relationship between CPUE and abundance, it will be important to determine the extent to which fishers' ability to locate the areas of greatest fish abundance interacts with habitat selection in fish.

Acknowledgements

The Killam Trust and the Natural Sciences and Engineering Research Council of Canada provided financial support for this work. We thank Don Ludwig, Mark Maunder, two anonymous referees, and an Associate Editor for very useful comments on an earlier version of this manuscript and participants of the Third Mote Symposium with whom we had useful discussions regarding this work.

References

Allen, R., and Punsly, R. 1984. Catch rates as indices of abundance of yellowfin tuna *Thunnus albacares* in the eastern Pacific Ocean. Inter-Am. Trop. Tuna Comm. Bull. **18**: 301–379.

Bannerot, S.P., and Austin, C.B. 1983. Using frequency distributions of catch per unit effort to measure fish-stock abundance. Trans. Am. Fish. Soc. **112**: 608–617.

Baranov, F.I. 1918. On the question of the biological basis of fisheries. Nauchn. Issled. Ikhtiologicheskii Inst. Izv. **1**: 81–128.

Beverton, R.J., and Holt, S.J. 1957. On the dynamics of exploited fish populations. Fish. Invest. Ser. II. Mar. Fish. G.B. Minist. Agric. Fish. Food, **19**: 533.

Bollen, K.A. 1989. Structural equations with latent variables. John Wiley & Sons, New York.

Cadigan, N. 1993. Structural equation models and small sample bias reduction with application to fishery data. M.Sc. thesis, Memorial University of Newfoundland, St. John's, Nfld.

Carlin, B.P., and Louis, T.A. 1996. Bayes and empirical Bayes methods for data analysis. Vol. 69 of Monographs on Statistics and Applied Probability. Chapman and Hall, London, U.K.

Clark, C.W., and Mangel, M. 1979. Aggregation and fishery dynamics: a theoretical study of schooling and the purse seine tuna fisheries. Fish. Bull. U.S. **77**: 317–337.

Cooper, H., and Hedges, L.V. 1994. The handbook of research synthesis. Russell Sage Foundation, New York.

Crecco, V., and Overholtz, W.J. 1990. Causes of density-dependent catchability for Georges Bank haddock *Melanogrammus aeglefinus*. Can. J. Fish. Aquat. Sci. **47**: 385–394.

Darby, C.D., and Flatman, S. 1994. Virtual population analysis: version 3.1 users guide. Information Technology Series No. 1. Directorate of Fisheries Research, Lowestoft, U.K.

DerSimonian, R., and Laird, N. 1986. Meta-analysis in clinical trials. Controlled Clin. Trials, **7**: 177–188.

Dunn, A., Harley, S.J., Doonan, I.J., and Bull, B. 2000. Calculation and interpretation of catch-per-unit-effort (CPUE) indices. N.Z. Fish. Assess. Rep. 2000/1. Ministry of Fisheries, Wellington, New Zealand.

Efron, B. 1996. Empirical Bayes methods for combining likelihoods. J. Am. Stat. Assoc. **91**: 538–565.

Fretwell, S., and Lucas, H. 1970. On the territorial behavior and other factors influencing the distribution in birds. I. Theoretical development. Acta Biotheor. **19**: 16–36.

Fuller, W.A. 1987. Measurement error models. John Wiley & Sons, New York.

Gillis, D.M., and Peterman, R.M. 1998. Implications of interference among fishing vessels and the ideal free distribution to the interpretation of CPUE. Can. J. Fish. Aquat. Sci. **55**: 37–46.

Hardy, R.J., and Thompson, S.G. 1996. A likelihood approach to meta-analysis with random effects. Stat. Med. **15**: 619–629.

Hilborn, R., and Walters, C.J. 1992. Quantitative fisheries stock assessment: choice, dynamics and uncertainty. Chapman and Hall, New York.

Hutchings, J.A. 1996. Spatial and temporal variation in the density of northern cod and a review of hypotheses for the stock's collapse. Can. J. Fish. Aquat. Sci. **53**: 943–962.

Liermann, M., and Hilborn, R. 1997. Depensation in fish stocks: a hierarchic Bayesian meta-analysis. Can. J. Fish. Aquat. Sci. **54**: 1976–1985.

MacCall, A.D. 1990. Dynamic geography of marine fish populations. University of Washington Press, Seattle, Wash.

Meyer, R., and Millar, R.B. 1999. Bayesian stock assessment using a state–space implementation of the delay difference model. Can. J. Fish. Aquat. Sci. **56**: 37–52.

Myers, R.A., and Cadigan, N.G. 1993. Density-dependent juvenile mortality in marine demersal fish. Can. J. Fish. Aquat. Sci. **50**: 1576–1590.

Myers, R.A., and Cadigan, N.G. 1995. Was an increase in natural mortality responsible for the collapse of northern cod? Can. J. Fish. Aquat. Sci. **52**: 1274–1285.

Myers, R.A., Bowen, K.G., and Barrowman, N.J. 1999. The maximum reproductive rate of fish at low population sizes. Can. J. Fish. Aquat. Sci. **56**: 2404–2419.

Paloheimo, J.E., and Dickie, L.M. 1964. Abundance and fishing success. J. Cons. Int. Explor. Mer, **155**: 152–163.

Peterman, R.M., and Steer, G.J. 1981. Relation between sport-fishing catchability coefficients and salmon abundance. Trans. Am. Fish. Soc. **110**: 585–593.

Punt, A., and Hilborn, R. 1997. Fisheries stock assessment and decision analysis: the Bayesian approach. Rev. Fish Biol. Fish. **7**: 35–65.

Quinn, T.J., and Deriso, R.B. 1999. Quantitative fish dynamics. Oxford University Press, New York.

Richards, L.J., and Schnute, J.T. 1986. An experimental and statistical approach to the question: is CPUE an index of abundance? Can. J. Fish. Aquat. Sci. **43**: 1214–1227.

Rose, G.A., and Leggett, W.C. 1991. Effects of biomass–range interactions on catchability of migratory demersal fish by mobile fisheries: an example of Atlantic cod (*Gadus morhua*). Can. J. Fish. Aquat. Sci. **48**: 843–848.

Sampson, D.B. 1990. Local catch per unit effort as an index of global abundance. *In* Catch effort sampling strategies: their application in freshwater fisheries management. *Edited by* I.G. Cowx. Fishing News Books, Oxford, U.K. pp. 275–284.

SAS Institute Inc. 1990. SAS Technical Report P-200, SAS/STAT software: CALIS and LOGISTIC procedures, release 6.04. SAS Institute Inc., Cary, N.C.

Searle, S.R., Casella, G., and McCulloch, C.E. 1992. Variance components. John Wiley & Sons, New York.

Seber, G.A.F. 1982. The estimation of animal abundance and related parameters. Macmillan Publishing Co., New York.

Swain, D.P., and Sinclair, A.F. 1994. Fish distribution and catchability: what is the appropriate measure of distribution? Can. J. Fish. Aquat. Sci. **51**: 1046–1054.

Walters, C., and Ludwig, D. 1994. Calculation of Bayes posterior probablility distributions for key population parameters. Can. J. Fish. Aquat. Sci. **51**: 713–722.

The use of marine reserves in evaluating the dive fishery for the warty sea cucumber (*Parastichopus parvimensis*) in California, U.S.A.

Stephen C. Schroeter, Daniel C. Reed, David J. Kushner, James A. Estes, and David S. Ono

Abstract: Management of sustainable fisheries depends upon reliable estimates of stock assessment. Assessment of many stocks is based entirely on fishery-dependent data (e.g., catch per unit effort), which can be problematic. Here we use fishery-independent data on stock size, collected within and outside of no-take reserves before and after the onset of fishing, to evaluate the status of the dive fishery for warty sea cucumbers, *Parastichopus parvimensis*, in southern California. Long-term monitoring data showed that abundance decreased throughout the Channel Islands within 3–6 years after the onset of fishing. No significant changes in the abundance of *P. parvimensis* were observed at the two non-fished reserve sites, although densities tended to increase following onset of the fishery. Before–after, control–impact (BACI) analyses of seven fished and two non-fished sites implicated fishing mortality as the cause of 33–83% stock declines. In sharp contrast, stock assessment based on CPUE data showed no declines and a significant increase at one island. To date, most discussion on marine reserves has focused on the protection and enhancement of exploited populations. Our study demonstrates the critically important, but often overlooked, role that marine reserves can play in providing reliable information on stock assessment.

Résumé : Une gestion durable des pêches nécessite des estimations fiables de l'évaluation des stocks. L'évaluation de nombreux stocks est basée entièrement sur des données provenant de la pêche commerciale (e.g. la capture par unité d'effort), ce qui peut poser un problème. Nous utilisons ici des données de taille du stock indépendantes de la pêche, récoltées à l'intérieur et à l'extérieur des zones protégées sans récolte, avant et après le début de la pêche, pour évaluer l'état de la pêche commerciale en plongée du concombre de mer verruqueux *Parastichopus parvimensis* dans le sud de la Californie. Des données provenant d'un suivi à long terme indiquent que la densité a diminué dans toute la région des îles de Santa Barbara en moins de trois à six ans après le début de la pêche. Aucun changement significatif de densité de *P. parvimensis* n'a été observé à deux réserves sans pêche, bien que les densités aient eu tendance à augmenter après le début de la pêche. Des analyses de type BACI (avant-après; témoin-impact) de sept sites de pêche et de deux sites non-exploités identifient la mortalité due à la pêche comme la cause de 33 à 83% du déclin des stocks. En revanche, les estimations de stock basées sur des données de CPUE ne révèlent aucun déclin et elles indiquent même une augmentation significative de densité à une des îles. Jusqu'à présent, la plupart des discussions sur les réserves marines ont traité de la protection et du redressement des populations exploitées. Notre étude démontre le rôle critique, mais souvent méconnu, que les réserves marines jouent en fournissant des données fiables sur l'estimation des stocks.

[Traduit par la Rédaction]

Introduction

Managing a sustainable fishery requires some means of assessing the current status of a population and how it changes over time. Only then can the impacts of fishing be evaluated and effective fishery management implemented. Typically, stock assessment is based on fishery-dependent data such as catch per unit effort (CPUE), which are much less expensive to obtain than direct data on stock size. The use of CPUE data to evaluate the status of exploited stocks can be problematic because of difficulties in standardizing effort (particularly in light of changing fishing technologies and catch efficiency) and the reluctance of some fishermen to accurately report their catch for fear of divulging trade secrets (Hillborn and Walters 1992).

Comparisons involving no-take marine reserves that make use of fishery-independent data (e.g., direct estimates of population abundance) offer an alternative approach to stock

Received February 21, 2001. Accepted July 4, 2001. Published on the NRC Research Press Web site at http://cjfas.nrc.ca on August 17, 2001.
J16232

S.C. Schroeter[1] and D.C. Reed. Marine Science Institute, University of California, Santa Barbara, CA 93106, U.S.A.
D.J. Kushner. Channel Islands National Park, 1901 Spinnaker Drive, Ventura, CA 93001, U.S.A.
J.A. Estes. United States Geological Survey, Institute of Marine Sciences, University of California, Santa Cruz, CA 95064, U.S.A.
D.S. Ono. California Department of Fish and Game, 1933 Cliff Drive, Suite 9, Santa Barbara, CA 93109, U.S.A.

[1] Corresponding author (e-mail: schroete@lifesci.ucsb.edu).

DOI: 10.1139/cjfas-58-9-1773

assessment. Numerous studies have reported higher abundances and larger sizes of fish inside no-take reserves relative to outside, while others have reported increases in the abundance and size of fish in an area following its designation as a no-take zone (reviewed in Rowley 1992; Halpern 2002). Such comparisons between impacted (i.e., fished) and control (i.e., non-fished) areas that are separated either in space or time are generally considered inadequate for assessing most environmental impacts, which are best evaluated by comparing population changes in impacted and non-impacted areas before and after the impact occurs (Underwood 1993; Stewart-Oaten and Bence 2001). Unfortunately, the use of a before–after, control–impact design (i.e., BACI) to evaluate impacts from fishing is not feasible for most established fisheries owing to a lack of data on stocks before fishing and (or) the lack of data from a suitable non-fished control site. Such may not be the case for emerging fisheries, however, and no-take reserves that use adequate monitoring programs offer a powerful means of assessing the status of harvested stocks and their likelihood for sustainability.

Here we take a BACI approach to evaluate the status of the recently developed dive fishery for the warty sea cucumber, *Parastichopus parvimensis*, in southern California. Fishery-independent data used in our analyses were obtained from long-term kelp forest monitoring programs conducted by the National Park Service (NPS) and the United States Geological Survey (USGS). These data consisted of estimates of *P. parvimensis* abundance in fished and non-fished areas before and after the onset of the fishery. As mentioned above, stock assessment based on fishery-dependent data is often suspect. To evaluate if this was true for the warty sea cucumber fishery, we compared results on the status of the fishery based on long-term population monitoring with those estimated by data on CPUE.

Methods

History of the fishery

Parastichopus parvimensis is a large sea cucumber that occurs from Baja California to Monterey Bay, California. It is most common south of Point Conception (Brumbaugh 1980) and lives in a variety of habitats from the intertidal zone to at least 30-m depth (Muscat 1983; Kalvass 1992). It is most abundant on shallow reefs where it feeds on organic detritus contained in thin layers of soft sediments that accumulate on the bottom. In 1982, an experimental dive fishery for *P. parvimensis* began in southern California. Like other large sea cucumbers, *P. parvimensis* is harvested for its body wall and musculature, which are dried and exported to Asian markets. Initially, fishing was sporadic, often consisting of a single day's catch by a single boat. Harvesting permits were required beginning in 1992 as the Asian demand for imported sea cucumbers steadily increased as a result of the overharvesting of traditional fishing grounds in the Indo-Pacific. The majority of the permit holders in southern California are also sea urchin fishermen and most of the landings for *P. parvimensis* take place from June through August when the commercial harvest of sea urchins is prohibited (D. Ono, unpublished data). The commercial fishery for *P. parvimensis* began in earnest in the mid-1990s and is largely unregulated (i.e., there are no restrictions on the size, season, or amount of harvest). Most of the catch is taken from the Channel Islands located off the coast of Santa Barbara, California.

Data on *P. parvimensis* abundance

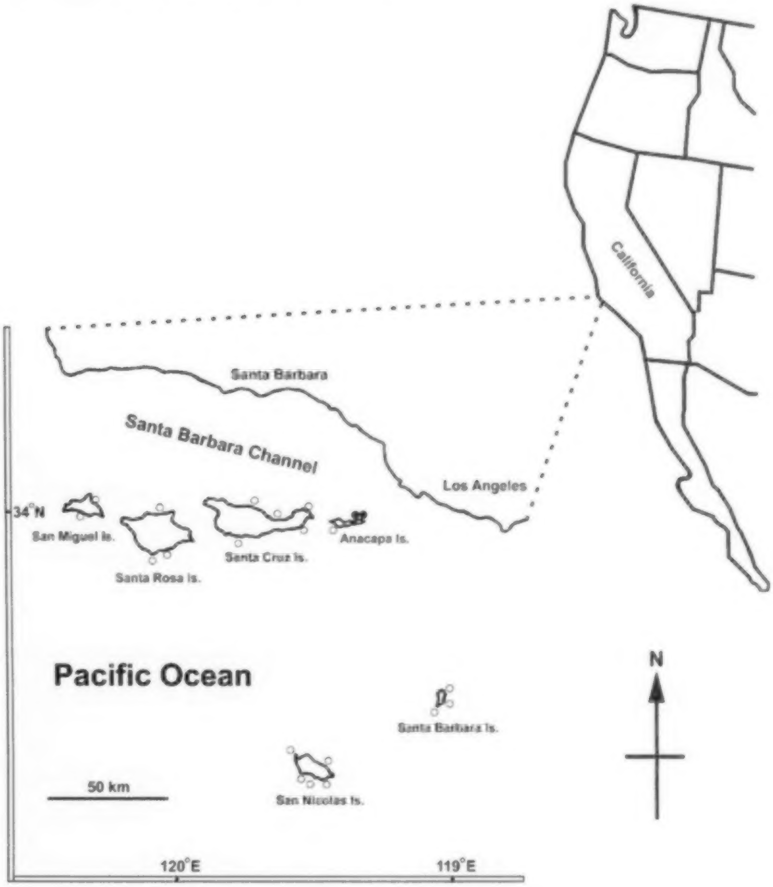
Data on *P. parvimensis* abundance used in our evaluation of the fishery were collected at the five northern Channel Islands (San Miguel Island (SMI), Santa Rosa Island (SRI), Santa Cruz Island (SCI), Anacapa Island (ANI), and Santa Barbara Island (SBI)) and at nearby San Nicolas Island (SNI) (Fig. 1). Approximately two-thirds of the *P. parvimensis* harvest in California has come from these six islands (California Department of Fish and Game (CDFG) CMASTR data). Data on *P. parvimensis* abundance from the northern Channel Islands were obtained from the NPS, which has collected data annually on the abundance of a wide variety of species that inhabit kelp forests at 16 sites around the five islands since 1982 (Davis et al. 1997). Two of the sites on the northeast side of ANI (Landing Cove and Cathedral Cove) are in a marine reserve where fishing has not been allowed since 1978. Fishing is permitted at the other 14 sites. *Parastichopus parvimensis* abundance at each of the 16 sites was estimated from non-destructive sampling of non-cryptic individuals. Data were collected once per year in the summer (June to August) by divers in 1 m^2 or 2 m^2 quadrats (n = 12 to 40 quadrats per site), which were randomly placed within a permanent 100 m × 20 m sampling area that defined each site. Sites for long-term monitoring were not specifically chosen by the NPS for the purpose of monitoring *P. parvimensis* abundance, but rather they were selected to represent the broad range of environmental conditions and biological assemblages in the park (Davis et al. 1997). Monitoring sites were generally located in areas of continuous reef with relatively low coverage of sand and cobble. The location and physical characteristics of each site are summarized in Reed et al. (2000).

Comparable data on *P. parvimensis* abundance for five sites off SNI were obtained from the USGS. The five sites were distributed around the island and were chosen by the USGS to represent the broad range of oceanographic conditions at SNI. The USGS has been conducting semi-annual (April and October) monitoring of the kelp forest communities at these sites since 1980. On each survey, *P. parvimensis* are counted by divers in five permanent 10 m × 2 m quadrats at each site using a non-destructive sampling procedure similar to that used by the NPS. We used only data from October surveys at SNI in our analyses to maintain compatibility with annual data from the northern Channel Islands. Fishing is permitted at all sites at SNI.

Before–after, control–impact (BACI) analyses

BACI analyses were done to estimate the effects of fishing on populations of *P. parvimensis* at the permanent monitoring sites on the six islands. The BACI analysis and its assumptions are described in detail elsewhere (Stewart-Oaten et al. 1986; Stewart-Oaten and Bence 2001) and are summarized briefly below. In a BACI design, control and impact sites are sampled concurrently on multiple dates before and after an impact (i.e., onset of fishing) occurs. Each survey date during the before period provides an estimate of the spatial difference between the impact and control sites. Continued sampling during the after period yields a time series of differences between the impact and control sites from the before and after periods. In our analyses, the impact was the commercial dive fishery for *P. parvimensis*. The start of the commercial fishery marked the beginning of the after period and was defined for each island as the year in which the cumulative catch equaled or exceeded 5% of the total cumulative catch through 1999. The no-take reserves at Landing Cove and Cathedral Cove on ANI served as our control (non-fished) sites, while all other sites were considered impact (i.e., fished) sites that varied in their intensity of fishing pressure. We used the mean annual abundance of *P. parvimensis* at Landing and Cathedral Coves as the control in all BACI analyses. The mean difference between the impact and control sites in the before period was compared statistically with that in the after period using a t test to determine if there has been an impact and the

Fig. 1. Map of the study area showing the locations of long-term monitoring sites where data on the abundance of *P. parvimensis* have been collected. Fishing has been prohibited since 1978 in the two sites at Anacapa Island designated by solid circles. Sites designated by open circles have had no restrictions on the *P. parvimensis* harvest.



relative size of its effect. Separate BACI analyses were done for each fished site. In all BACI analyses, t tests were one-tailed to test the null hypothesis that there was no decline in the abundance of *P. parvimensis* following the onset of commercial fishing.

Some of the underlying assumptions of the BACI model are (*i*) the data are additive (i.e., the effects of location (fished vs. non-fished) and period (before vs. after the onset of fishing) are constant and can be added to the underlying mean abundance to obtain the expected abundances in the different cells of the design); (*ii*) the random errors in the differences between fished and non-fished sites are independent; and (*iii*) the random errors are normally distributed with equal variances and means of zero. BACI analyses were performed only on those sites for which data met these assumptions. The statistical procedures that we used to test these assumptions are described in Stewart-Oaten (1987), Carpenter (1989), and Stewart-Oaten et al. (1992).

Absolute changes in the abundance of *P. parvimensis* following the onset of fishing were evaluated with t tests that compared the average abundance of *P. parvimensis* in the before and after periods. Such before–after t tests were done for all 19 fished sites and the average of the two non-fished control sites. In all before–after comparisons, t tests were two-tailed to test the null hypothesis of no change in the abundance of *P. parvimensis* following the onset of commercial fishing.

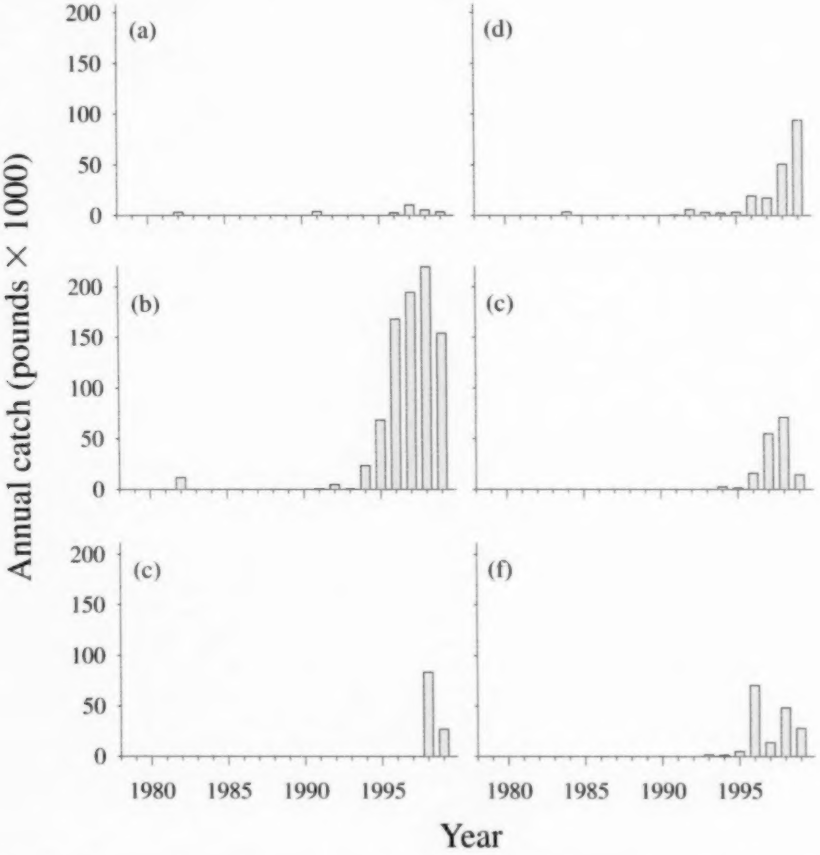
Data on CPUE

Data on CPUE were obtained from the CDFG CMASTR database and represent catch (in pounds) per boat per day. The data are based on landings weighed at the dock by the processor, who records the data on a "fish ticket" which is given to the CDFG. Fishermen are required to assign their landings to 10 × 10 nautical mile fish blocks and report the fishing effort (i.e., number of boat days) allocated to their catch. Because of the relatively low spatial resolution of these data, CPUE could only be estimated at the scale of islands and not sites within islands. The CMASTR data used in our analyses of CPUE covered the entire after period for each of the six islands.

Results

As mentioned above (see Methods), we chose the first year in which the cumulative catch on each island equaled or exceeded 5% of the total cumulative catch as the beginning of the after period in our BACI analyses. This occurred in 1993 for SCI and SRI, 1995 for SMI, ANI, and SNI, and 1997 for SBI (Fig. 2). As of 1999, approximately 56% of the total cumulative catch taken from all six islands was taken

Fig. 2. Annual catch of warty sea cucumbers (*P. parvimensis*) by island at five northern Santa Barbara Channel Islands and San Nicolas Island: (*a*) San Miguel Island; (*b*) Santa Cruz Island; (*c*) Santa Barbara Island; (*d*) Santa Rosa Island; (*e*) Anacapa Island; and (*f*) San Nicolas Island.



from SCI, followed by 13% from SRI, 11% from each of SNI and ANI, 7% from SBI, and <2% from SMI.

Monitoring data suggest that the abundance of *P. parvimensis* decreased throughout the islands at fished sites following the onset of fishing (Table 1). Within 3 to 6 years of the start of the fishery, significant declines in sea cucumber abundance (ranging from 25% to 100%) occurred at 8 of 19 sites spread among five islands. An additional seven sites showed non-significant (i.e., $P > 0.05$) reductions in sea cucumber abundance, while four sites showed non-significant increases. In contrast, there was a trend of increasing abundance of *P. parvimensis* at the two non-fished reserve sites after 1993, 1995, and 1997, which marked the onset of fishing at SCI and SRI, ANI, SNI, and SMI, and SBI, respectively.

Only 7 of the 19 fished sites met the underlying assumptions of BACI (Table 2). The use of BACI to estimate impacts from fishing was excluded at nine sites because the data in the before period were non-additive. Non-additivity can be expected in cases where abundance at the impact site is consistently much lower than at the control site. Under such conditions, the difference between impact and control sites will be much greater during years when sea cucumber abundance is generally high compared with years when sea cucumber abundance is generally low. Such was the case for eight of the nine sites that failed to meet the assumption of additivity. These eight sites were probably not subjected to intensive fishing pressure because of their inherently low sea cucumber stocks, and five of them showed no significant difference in sea cucumber abundance between the before and after periods (in all cases, $P > 0.10$, Table 1). Pelican Bay on Santa Cruz Island was the lone exception to this rule as data from this site were non-additive despite relatively high abundances in the before period. The high densities of *P. parvimensis* at Pelican Bay and its close proximity to mainland ports make it an attractive site for fishing. The significant 65% decline in sea cucumber abundance observed at this site from the before to after periods (Table 1) coupled with the high landings reported for Santa Cruz Island (Fig. 2) suggest that it was probably heavily fished. Three additional sites with relatively high densities of *P. parvimensis* (Nav Fac, Admiral's Reef, and Gull Island) were excluded from analyses using BACI because they showed positive trends in abundance during the before period (Table 2).

All seven sites that met the underlying assumptions of BACI showed significant declines (relative to non-fished sites) following the onset of fishing (Table 3, Fig. 3). The

Table 1. Percentage change in the mean annual abundance of warty sea cucumbers (*P. parvimensis*) at the 19 fished and 2 non-fished island sites following the onset of fishing.

Island	Site	Onset of fishing	% change	*t* value	*P*
	Fished				
San Nicolas	West Dutch Harbor	1995	–47	–3.1	**0.007**
	East Dutch Harbor	1995	–3	–0.2	0.857
	West End	1995	–24	–1.3	0.209
	Nav Fac	1995	+31	0.7	0.519
	Daytona Beach	1995	+12	0.8	0.452
Santa Barbara	Arch Point	1997	–48	–1.6	0.128
	Cat Canyon	1997	+52	2.0	0.071
	SE Sea Lion Rookery	1997	–31	–2.7	**0.017**
Anacapa	Admiral's Reef	1995	+18	1.4	0.171
Santa Cruz	Scorpion Anchorage	1993	–62	–3.0	**0.009**
	Pelican Bay	1993	–73	–3.4	**0.002**
	Fry's Harbor	1993	–77	–7.8	**<0.002**
	Yellowbanks	1993	–37	–1.9	0.082
	Gull Island	1993	–34	–1.3	0.225
Santa Rosa	Rodes Reef	1993	–100	–3.0	**0.010**
	Johnson's Lee North	1993	–49	–3.5	**0.003**
	Johnson's Lee South	1993	–9	–0.2	0.867
San Miguel	Hare Rock	1995	–70	–2.5	**0.026**
	Wyckoff Ledge	1995	–10	–0.5	0.649
	Non-fished				
Anacapa	Landing and Cathedral Coves	1993	87	0.5	0.648
		1995		2.1	0.052
		1997		1.1	0.272

Note: Onset of fishing is defined as the first year that the total cumulative catch at an island equaled or exceeded 5%. Percentage change at the non-fished sites was based on an average of Landing and Cathedral Coves. Because the onset of fishing varied among islands, the percentage change in *P. parvimensis* abundance at Landing and Cathedral Coves was calculated for three different time periods to allow comparisons with sites having different onsets of fishing. *P* values < 0.05 are bolded.

Table 2. Results for tests of additivity and no trends in the data on the annual abundance of warty sea cucumbers (*P. parvimensis*) at 19 island sites subjected to fishing, collected before the onset of fishing.

Island	Fished site	Additivity	No trends	Abundance prior to fishing
San Nicolas	**West Dutch Harbor**	**Yes**	**Yes**	**5.36**
	East Dutch Harbor	No	Yes	2.27
	West End	No	Yes	1.68
	Nav Fac	Yes	No (+)	5.69
	Daytona Beach	No	Yes	1.45
Santa Barbara	Arch Point	No	Yes	2.39
	Cat Canyon	No	Yes	3.02
	SE Sea Lion Rookery	**Yes**	**Yes**	**9.28**
Anacapa	Admiral's Reef	Yes	No (+)	12.47
Santa Cruz	**Scorpion Anchorage**	**Yes**	**Yes**	**6.83**
	Pelican Bay	No	Yes	10.67
	Fry's Harbor	**Yes**	**Yes**	**21.11**
	Yellowbanks	**Yes**	**Yes**	**7.50**
	Gull Island	Yes	No (+)	10.28
Santa Rosa	Rodes Reef	No	Yes	0.23
	Johnson's Lee North	**Yes**	**Yes**	**5.23**
	Johnson's Lee South	**Yes**	**Yes**	**1.74**
San Miguel	Hare Rock	No	Yes	1.72
	Wyckoff Ledge	No	Yes	2.05

Note: "Yes" indicates that the data for a given site passed the underlying assumption of BACI and "No" indicates that the data failed the underlying assumption of BACI. (+) indicates trend was positive. BACI tests to evaluate the effects of fishing on the abundance of *P. parvimensis* were done for sites that passed tests for both additivity and no trends (shown in bold). Abundance prior to fishing is the mean annual density of *P. parvimensis* (number·10 m^{-2}) in the before period.

Table 3. Relative changes in the density of warty sea cucumbers (*P. parvimensis*) following the onset of commercial fishing at seven island sites.

Island	Site	Relative % change	df	*t* value	*P*
San Nicolas	West Dutch Harbor	–67	15	–5.3	<0.001
Santa Barbara	SE Sea Lion Rookery	–52	16	–1.7	0.027
Santa Cruz	Scorpion Anchorage	–76	16	–3.8	<0.001
	Fry's Harbor	–83	7	–4.9	<0.001
	Yellowbanks	–53	12	–2.1	0.015
Santa Rosa	Johnson's Lee South	–33	16	–1.4	0.044
	Johnson's Lee North	–51	16	–2.9	0.003

Note: Relative changes represent the difference in sea cucumber density between a given fished site and the mean of non-fished control sites in the before period relative to the difference between the fished and non-fished sites in the after period. Separate BACI *t* tests that compared the average deltas (fished – non-fished) from the before and after periods were used to determine the statistical significance of relative change at each site. *t* tests are one-tailed (testing for declines at fished sites).

decline in the relative abundance of *P. parvimensis* at these sites (as estimated by BACI) ranged from 33% to 83%. The smallest relative decline occurred at Johnson's Lee South, which supported sparse densities of *P. parvimensis* compared with the other six sites evaluated using BACI (Table 2 and Fig. 3). This was the only one of the seven sites that did not show a significant decline in the absolute density of *P. parvimensis* from the before to after period (Table 1). The absolute density of *P. parvimensis* at the other six sites declined by 25% to 67% following the onset of fishing.

An evaluation of *P. parvimensis* stocks based on analyses using fishery-dependent data contrasted sharply with that based on BACI and before–after analyses of fishery-independent data. CPUE did not decline at any of the six islands during the 3- to 6-year period following the onset of fishing despite a general decline in *P. parvimensis* abundance during this time (Fig. 4). Surprisingly, Santa Rosa Island actually showed a significant increasing trend in CPUE.

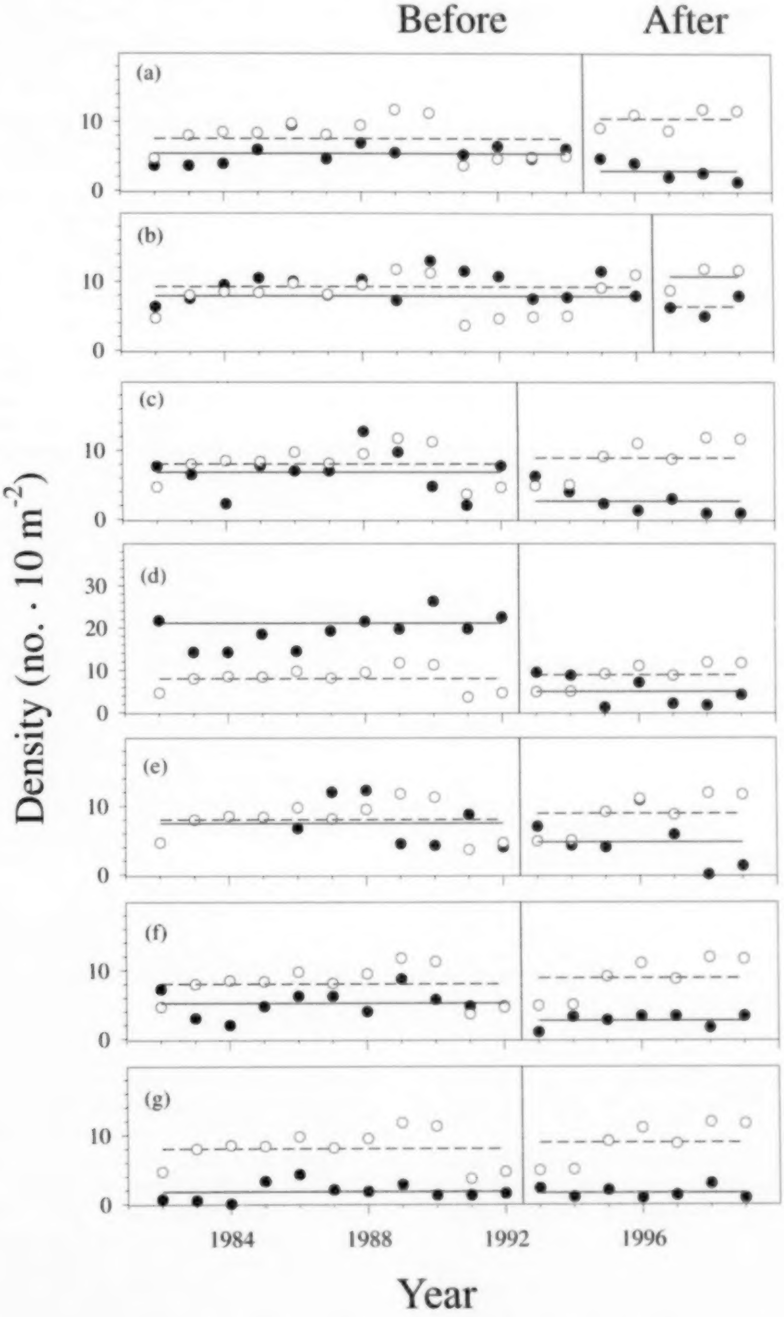
Discussion

Data on the abundance of *P. parvimensis* collected from the NPS and the USGS show that substantial and statistically significant population declines occurred in the Channel Islands within 3 to 6 years of the rapid expansion of the commercial dive fishery. The BACI analyses done on seven sites implicate fishing as a significant cause of these declines. An explicit assumption in our BACI analyses was that no fishing occurred in the two no-take zones on Anacapa Island, which were used as control sites. It is worth noting that any poaching done at these sites would have biased the BACI analyses and led to an underestimate of the impact of fishing. Thus, in this sense, the relative declines in sea cucumber abundance identified by BACI should be viewed as being conservative.

Regulations for most fisheries (including the dive fishery for *P. parvimensis*) require fishermen to report the amount, location (i.e., fish block), and date of their catch. These data are frequently used to calculate CPUE, which is used as an indicator of stock size. The expectation is that CPUE should decline with decreases in stock size. Our analyses show this not to be the case for *P. parvimensis* populations at the Channel Islands. CPUE for *P. parvimensis* either showed little change or significantly increased (e.g., Santa Rosa Island) despite general declines in sea cucumber abundance as indicated by the fishery-independent monitoring data. The specific reasons for the lack of correspondence between CPUE and stock size in this case are not known. The pattern of CPUE declining more slowly than abundance has been reported elsewhere by Hillborn and Walters (1992) who attributed it to two factors: (*i*) spatially structured stocks with substocks of differing densities and differing catchability and (*ii*) changes in catchability over time. Both factors appear to apply to the *P. parvimensis* fishery at the Channel Islands. Data collected prior to the fishery show densities of *P. parvimensis* differed greatly among sites and islands. Moreover, it is reasonable to assume that fishermen have become more efficient at locating and harvesting *P. parvimensis* over the recent history of the fishery, resulting in changes in catchability over time. Other factors could have lead to a constant CPUE in the face of declining stock size as well. For instance, search and handling times by fishermen might remain constant over a range of high stock densities. Furthermore, small vessels with limited holding capacity would tend to stabilize CPUE over a range of stock densities, especially where travel time between the port and fishing grounds is significant. Good correspondence between changes in CPUE and stock size may in fact only occur at low stock densities, where search time becomes a relatively large component of fishing effort.

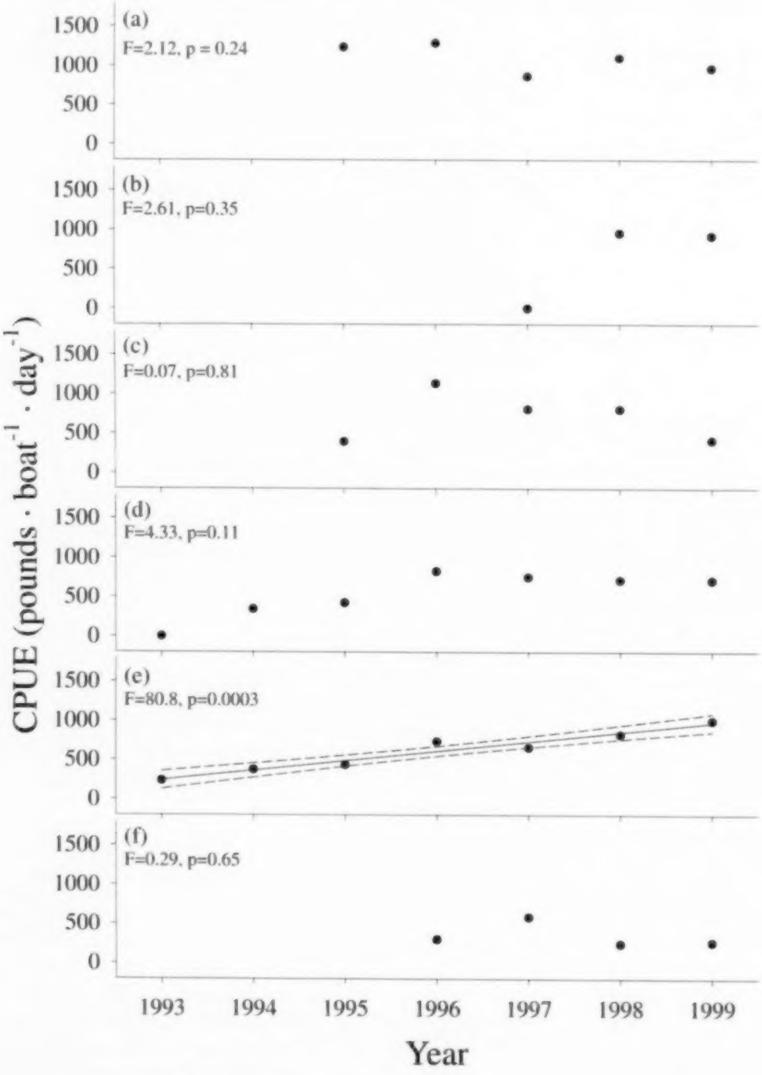
There are some limitations to using long-term monitoring data in fishery management. Foremost among these is that few long-term monitoring programs have been designed specifically for the purpose of fishery evaluation. Often, data collected by entities not associated with the fishing industry (e.g., universities, non-fishery agencies, private industry, etc.) are the only fishery-independent data available for a given stock. The locations, dates, and sampling frequency at which such data are collected are often suboptimal for evaluating the effects of fishing on stock size, particularly if BACI analyses are desired (e.g., McClanahan 1989). Such was the case for *P. parvimensis* in California. The only data on the long-term abundance of *P. parvimensis* are those of the NPS and the USGS. The monitoring programs of these two agencies were established primarily to track long-term changes in kelp forest communities at a wide array of sites that were selected to reflect the broad range of environmental conditions and biological assemblages at the Channel Islands. Consequently, only 7 of 19 sites met the statistical assumptions of our BACI analy-

Fig. 3. Densities of warty cucumbers (*P. parvimensis*) at fished sites on San Nicolas ((*a*) West Dutch Harbor), Santa Barbara ((*b*) Southeast Sea Lion Rookery), Santa Cruz ((*c*) Scorpion Anchorage, (*d*) Fry's Harbor, and (*e*) Yellowbanks), and Santa Rosa Islands ((*f*) Johnson's Lee North and (*g*) Johnson's Lee South). The annual mean for each fished site (solid circles) is plotted with the annual mean of the two non-fished reserve sites on Anacapa Island (open circles). Horizontal lines are means for fished (solid) and non-Fished (dotted) sites for the before and after periods (separated by vertical line).



ses for *P. parvimensis*. Nonetheless, an important advantage of these monitoring programs with respect to their use in evaluating the *P. parvimensis* fishery is that they began in advance of the fishery. Typically, monitoring programs are not begun until there is a perceived problem with an exploited population.

Fig. 4. Annual CPUE data of warty cucumbers (*P. parvimensis*) during the after period at six islands: (*a*) San Nicolas Island; (*b*) Santa Barbara Island; (*c*) Anacapa Island; (*d*) Santa Cruz Island; (*e*) Santa Rosa Island; and (*f*) San Miguel Island. Shown are *F* and *P* values for tests of significance of linear trends in CPUE vs. Time. For Santa Rosa Island, where the *P* value is <0.05, the linear fit (solid line) and upper and lower 95% confidence intervals (dotted lines) are shown.



Another limitation in using BACI has to do with assigning causality. Although the BACI analysis avoids the pitfalls of pseudoreplication (sensu Hurlbert 1984) associated with before–after or control–impact studies, it nonetheless is not a manipulative experiment and cannot completely rule out coincidental time by location interactions that coincide with the onset of the impact (Stewart-Oaten and Bence 2001). Schroeter et al. (1993) recognized this fact and addressed it by considering information in addition to BACI analyses that could either corroborate or lead to rejection of conclusions based on the BACI alone. The present study provides such additional information. The criteria used by the NPS and the USGS to select locations for long-term monitoring resulted in a dataset consisting of a large number of widely distributed sites that varied greatly in their abundance of *P. parvimensis* as well as in their distance from fishing ports. Such conditions undoubtedly resulted in a wide range of fishing pressures, which could be useful in isolating the effects of fishing (Pfister and Bradbury 1996; Kalvass and Hendrix 1997). In our study, sites closest to mainland ports (and coincidentally closest to the non-fished reserved sites) with high abundances of *P. parvimensis* in the before period (e.g., sites on SCI) were heavily fished and consequently suffered some of the greatest declines. By contrast, sites far from port (and coincidentally, far from non-fished reserve sites) with relatively low densities of sea cucumbers (e.g., sites on SMI and several on SNI) were fished less intensively and showed little change in the abundance of

P. parvimensis from the before to the after periods. Such patterns corroborate the results of our BACI analyses and provide additional evidence that implicates fishing as a major cause of declining stocks of *P. parvimensis* at the Channel Islands. Moreover, the spatial pattern of larger declines at fished sites closer to the non-fished control sites argues against the alternative hypothesis that the relative declines at fished sites were caused by site-specific oceanographic conditions in the after period that were favorable only to *P. parvimensis* in the vicinity of the control sites.

The use of no-take marine reserves to enhance depleted stocks has been the subject of much discussion and debate (Davis 1989; Dugan and Davis 1993; NRC 2001). The use of reserves for purposes of stock assessment, however, has received far less attention (NRC 2001). Our study demonstrates the value of an established marine reserve in assessing the status of the recent and largely unregulated dive fishery for *P. parvimensis*, a relatively sedentary sea cucumber. In the absence of a no-take reserve and long-term monitoring, catch data would have provided the only means for assessing *P. parvimensis* stocks in California. Our analyses show that management of the *P. parvimensis* fishery based solely on CPUE would lead to an inaccurate assessment of the stock, which could lead to the demise of the fishery. No-take reserves may also prove valuable in assessing the stock of established fisheries. Comparisons of stock abundance between protected and non-protected areas before and after a reserve is established can provide valuable information on the extent to which a fishery has caused stocks to decline, and on the resiliency of exploited stocks to recover in the absence of fishing. Whether used to evaluate fisheries with a long or short history, the value of no-take reserves to fishery managers is critically dependent on rigorous enforcement of no-take regulations, and on comprehensive long-term monitoring. Future plans involving the establishment of no-take zones for purposes of fishery enhancement should recognize the value of reserves in stock assessment and require the enforcement and monitoring that is needed to successfully achieve this important but often overlooked objective.

Acknowledgements

We thank R. Warner for insightful comments and discussion, D. Huang for assistance with graphics, and M. Kenner for making available the data from San Nicolas Island. This study was funded by the National Marine Fisheries Service (NOAA) under the Saltonstall–Kennedy Program (Award No. NA86FD0070) and the National Science Foundation (Award No. OCE-9982105)..

References

Brumbaugh, J.H. 1980. Holothuroidea: the sea cucumbers. *In* Intertidal invertebrates of California. *Edited by* R.H. Morris, D.P. Abbott, and E.C. Haderlie. Stanford University Press, Palo Alto. pp. 136–145.

Carpenter, S.J. 1989. Replication and treatment strength in whole lake experiments. Ecology, **70**: 453–463.

Davis, G.E. 1989. Designated harvest refugia: the next stage of marine fishery management in California. Cal. Coop. Oceanic Fish. Invest. Rep. **30**: 53–58.

Davis, G.E., Kushner, D.J., Mondragon, J.M., Mondragon, J.E., Lerma D., and Richards, D. 1997. Kelp forest monitoring handbook. Vol. 1. Sampling protocol. Channel Islands National Park, Ventura, Calif.

Dugan, J.E., and Davis, G.E. 1993. Applications of marine refugia to coastal fisheries management. Can. J. Fish. Aquat. Sci. **50**: 2029–2042.

Halpern, B. 2002. The impact of marine reserves: do reserves work and does reserve size matter? Ecol. Appl. **12**. In press.

Hillborn, R., and Walters, C.J. 1992. Quantitative fisheries stock assessment: choice, dynamics and uncertainty. Chapman and Hall. New York.

Hurlbert, S.J. 1984. Pseudoreplication and the design of ecological field experiments. Ecol. Monogr. **54**: 187–211.

Kalvass, P. 1992. Sea cucumbers. *In* California's living marine resources and their utilization. *Edited by* W.S. Leet, C.M. Dewees, and C.W. Haugen. California Sea Grant Extension Publication UCSGEP-92-12, Davis, Calif. pp. 44–45.

Kalvass, P.E., and Hendrix, J.M. 1997. The California red sea urchin, *Strongylocentrotus franciscanus*, fishery: catch, effort and management trends. Mar. Fish. Rev. **59**: 1–17.

McClanahan, T.R. 1989. Kenyan coral reef-associated gastropod fauna: a comparison between protected and unprotected reefs. Mar. Ecol. Progr. Ser. **53**: 11–20.

Muscat, A.M. 1983. Population dynamics and the effect on the infauna of the deposit-feeding holothurian P*arastichopus parvimensis* (Clark). Ph.D. dissertation, University of Southern California, Los Angeles, Calif. (USCSG-TD-02–83).

National Research Council, Committee on the Evaluation, Design, and Monitoring of Marine Reserves and Protected Areas in the United States, Ocean Studies Board, Commission on Geosciences, Environment, and Resources. 2001. Monitoring, research, and modeling. National Academy Press, Washington, D.C.

Pfister, C.A., and Bradbury, A. 1996. Harvesting red sea urchins: recent effects and future predictions. Ecol. Appl. **6**: 298–310.

Reed, D.C., Raimondi, P.T., Carr, M.H., and Goldwasser, L. 2000. The role of dispersal and disturbance in determining spatial heterogeneity in sedentary kelp-forest organisms. Ecology, **81**: 2011–2026.

Rowley, R.J. 1992. Impacts of marine reserves on fisheries: a report and review of the literature. Science and Research Series. Vol. 51. Department of Conservation, Wellington, New Zealand.

Schroeter, S.C., Kastendiek, J.D.D., Smith, R.O., and Bence, J.R. 1993. Detecting the ecological effects of environmental impacts: a case study of kelp forest invertebrates. Ecol. Appl. **3**(2): 331–350.

Stewart-Oaten, A. 1987. Assessing effects on fluctuating populations: tests and diagnostics. *In* ASA/EPA conferences on interpretation of environmental data. III. Sampling and site selection in environmental studies (May 14–15, 1987). Publication EPA-230-08-88-035, U.S. EPA Office of Policy, Planning and Evaluation, Washington, DC 20460.

Stewart-Oaten, A., and Bence, J.R. 2001. Temporal and spatial variation in environmental impact assessment. Ecol. Monogr. **71**: 305–337.

Stewart-Oaten, A., Murdoch, W.W., and Parker, K.E. 1986. Environmental impact assessment: "pseudoreplication" in time? Ecology, **67**: 929–940.

Stewart-Oaten, A., Bence, J.R., and Osenberg, C.W. 1992. Assessing effects of unreplicated perturbations: no simple solutions. Ecology, **67**: 929–940.

Underwood, A.J. 1993. On beyond BACI: sampling designs that might reliably detect environmental disturbances. Ecol. Appl. **4**: 3–15.

Evaluating sampling sufficiency in fish assemblage surveys: a similarity-based approach

Yong Cao, David P. Larsen, and Robert M. Hughes

Abstract: The number and identity of fish species occurring at a site at a particular time provide basic information for assessing biological integrity, inferring fish assemblage – environment relationships, and determining biodiversity patterns. Conclusions are often dependent on how sufficiently species richness and composition of fish assemblages are characterized by sampling. The proportion of total species richness obtained in a sample is an explicit measure of sampling sufficiency. However, because total species richness (TSR_{tru}) at a site is often unknown, sampling sufficiency cannot be determined directly. To overcome this difficulty, we developed a new approach, which is based on a relationship between the proportion of TSR_{tru} or $\%TSR_{tru}$ and the similarity among replicate samples (autosimilarity). With autosimilarity measured with the Jaccard coefficient (JC), a simple relationship was established: $\%TSR_{tru} = 100JC$. Fourteen sites where TSR_{tru} was reached or approached during sampling were selected from four surveys to validate this relationship. We used the approach to estimate the sample sizes required for 90, 95, and 100% TSR_{tru}, indicating that widely differing sampling efforts among sites are needed to obtain the same proportion of the local species pool. The results strongly support the use of the new approach in evaluating sampling sufficiency in stream and river fish surveys.

Résumé : Le nombre et l'identité des espèces de poissons présentes à un site à un moment donné sont les informations de base qui servent à évaluer l'intégrité biologique, à déterminer les relations des peuplements de poissons avec leur environnement et à décrire la structure de la biodiversité. Les conclusions que l'on tire dépendent donc souvent de la précision avec laquelle on a pu établir par échantillonnage la richesse en espèces et la composition des peuplements de poissons. La proportion de la richesse spécifique totale obtenue par échantillon est une mesure explicite de la suffisance de l'échantillonnage. Cependant, parce que la richesse spécifique totale (TRS_{tru}) à un site est souvent inconnue, la suffisance de l'échantillonnage ne peut être déterminée directement. Pour résoudre ce problème, nous avons exploré une nouvelle approche qui se base sur la relation entre la proportion de TRS_{tru}, ou le $\%TRS_{tru}$, et la similarité entre des échantillons répétés (autosimilarité). À l'aide du coefficient de similarité de Jaccard (JC), une relation simple a pu être établie : $\%TRS_{tru} = 100JC$. Quatorze sites tirés de quatre inventaires dans lesquels TRS_{tru} avait été déterminé ou approché par échantillonnage ont été retenus pour valider la relation. Nous avons ainsi estimé les tailles d'échantillons requises pour obtenir 90, 95 et 100% de TRS_{tru}; d'un site à un autre, des efforts très différents d'échantillonnages sont nécessaires pour obtenir une même précision de l'estimation du pool d'espèces locales. Nos résultats soulignent fortement la nécessité d'adopter une nouvelle approche pour évaluer la suffisance de l'échantillonnage dans les inventaires de poissons des rivières et des ruisseaux.

[Traduit par la Rédaction]

Introduction

Fish assemblage surveys are conducted mainly for three purposes: detection of the spatial–temporal patterns of species diversity and community structure in relation to environmental factors (e.g., Jackson and Harvey 1989; Benson and Magnuson 1992; Maret et al. 1997), assessment of biological integrity (Karr 1991; Hughes et al. 1998; Barbour et al. 1999), and conservation of biodiversity (Allan and Flecker 1993; Angermeier and Winston 1999; Whittier and Kincaid 1999). A critical aspect of assemblage surveys is sampling sufficiency, on which conclusions are often dependent. As with other taxa, determining sampling sufficiency is challenging in fish surveys. An ideal option for determining sampling sufficiency is to sample until species accumulation curves level (Gauch 1982; Kent and Coker 1992). This approach is practical when species richness is low and habitats are relatively homogeneous. However, spe-

Received December 6, 2000. Accepted June 15, 2001. Published on the NRC Research Press Web site at http://cjfas.nrc.ca on August 17, 2001.
J16122

Y. Cao.[1] NRC Research Associate, c/o Western Ecology Division, NHEERL-USEPA, 200 SW 35th Street, Corvallis, OR 97333, U.S.A.
D.P. Larsen. Western Ecology Division, NHEERL-USEPA, 200 SW 35th Street, Corvallis, OR 97333, U.S.A.
R.M. Hughes. Dynamac International, Inc., c/o USEPA Western Ecology Division, 200 SW 35th Street, Corvallis, OR 97333, U.S.A.

[1] Corresponding author at Department of Fisheries and Wildlife, Utah State University, Logan, UT 84322-5210, U.S.A. (e-mail: yong.cao@cnr.usu.edu).

Can. J. Fish. Aquat. Sci. **58**: 1782–1793 (2001)   DOI: 10.1139/cjfas-58-9-1782

cies richness varies widely over space and the spatial distribution of species is often patchy. Consequently, species curves often do not level at a practical amount of sampling effort.

A second option is to establish an empirical relationship between species richness and sampling effort by extensively sampling a limited number of sites, assuming that the relationship observed holds for a much larger area (Lyons 1992; Angermeier and Smogor 1995; Patton et al. 2000). This approach requires an accurate estimation of the total number of species (TSR_{tru}), which is often not reached even with extensive sampling. As it is often impractical to capture all species in routine sampling, a third option is to estimate the sampling effort required for obtaining a large proportion of the TSR_{tru} (e.g., 90% TSR_{tru}). $\%TSR_{tru}$ indicates what proportion of all species at a site is included in the comparisons of species composition among sites and can be considered as an explicit measure of sampling sufficiency. The sampling effort required for obtaining either TSR_{tru} or a specified $\%TSR_{tru}$ also varies substantially both among regions and within the same region (Angermeier and Smogor 1995). Thus, a standard sampling effort may oversample at some sites but undersample at others. It is highly desirable to determine the sample size for a specified level of $\%TSR_{tru}$ for each site.

The underlying reason for high variability in sampling effort required for the same level of $\%TSR_{tru}$ has been related to biological and habitat heterogeneity (see MacIntosh 1991 for definitions) and related to sampling efficiency (Bayley et al. 1989; Lyons 1992; Angermeier and Smogor 1995). In order to obtain a particular level of $\%TSR_{tru}$, biological and habitat heterogeneity should be considered. Cao et al. (in press) used the average similarity among replicate samples randomly drawn from a site (i.e., autosimilarity) to indicate the level of heterogeneity and to measure sampling sufficiency. When biological or habitat heterogeneity or both are high, replicates are less similar and vice versa.

The objectives of this study are to (*i*) develop a conceptual framework and a simple mathematical model that relates the proportion of total species richness observed (TSR_{obs}) and total species richness (TSR_{tru}) to autosimilarity, (*ii*) test the new model and related assumptions, (*iii*) use the model to estimate the sampling effort required for collecting 90, 95, and 100% TSR_{tru} (local species pool) in several case studies, and (*iv*) discuss the technical implications and potential limitations of the model and illustrate how it can be used to determine sampling sufficiency in the field.

Methods and data sets

Conceptual approach

We base our analysis on an examination of a relationship between total species richness at a site and autosimilarity. We use a binary similarity index, the Jaccard coefficient (JC), as the measure of similarity because we are interested in the number of species present without regard to relative abundance. The JC is widely accepted in community studies (Legendre and Legendre 1998) and easy to calculate in the field.

Sampling fish assemblages in streams and rivers is often accomplished by collecting at specified sites with the site subdivided into smaller areas such as habitat units or transects. For example, at a particular site, sampling might occur at 10 transects with the fish collected in each transect recorded separately, or sampling might occur sequentially in habitat units such as pools or riffles and each sample from a pool or riffle recorded separately. For the present purpose, we considered each of these separate sample units as a replicate, or the mathematical combination of multiple units as a replicate. For example, four transects can be randomly drawn from a total of 10 transects and combined into one composite replicate. Sample size refers to the number of sample units (habitat units, transects) pooled and is exchangeable with sampling effort in this study. Sample size can also be expressed as the number of wetted channel widths sampled.

A common observation in assemblage surveys is that the number of species collected increases with sampling effort, initially rapidly and then slowly. When the curves flatten, we are reasonably certain that all of the species present have been sampled and use this as an estimate of total species richness (TSR_{tru}). If the curves do not flatten, total species richness observed across all sample units (TSR_{obs}) is usually an underestimate of TSR_{tru}. The average richness per replicate ($\overline{SR}$) can be expressed as the percentage of either TSR_{tru} or TSR_{obs}:

$$(1) \qquad \%TSR_{tru} = \frac{\overline{SR}}{TSR_{tru}} \cdot 100$$

$$(2) \qquad \%TSR_{obs} = \frac{\overline{SR}}{TSR_{obs}} \cdot 100$$

The $\%TSR_{obs}$ is larger than $\%TSR_{tru}$ when TSR_{obs} is an underestimate of TSR_{tru}. A mathematical relationship linking TSR_{tru}, TSR_{obs}, and JC can be developed as follows.

Relating TSR_{obs} to JC

When an even number of sample units (N) is collected, the sample units can be divided into two replicates, each replicate consisting of the pool of species in $0.5N$ sample units. The N here should not be too small in order to avoid the case where the two replicates are distinct or identical just by chance. The JC is calculated as follows:

$$(3) \qquad JC = \frac{c}{a + b + c}$$

where a is the number of species unique to replicate 1, b is the number of species unique to replicate 2, and c is the number of species common to both replicates.

The total number of species in the two replicates is

$$(4) \qquad TSR_{obs} = a + b + c$$

The average species richness per replicate is

$$(5) \qquad \overline{SR} = 0.5(a + b) + c$$

We incorporated eqs. 4 and 5 into eq. 2 and then simplified using eq. 3 as follows:

$$(6) \qquad \%TSR_{obs} = 100 \cdot \frac{0.5(a + b) + c}{a + b + c} = 50 + 50\,JC$$

This relationship states that the average number of species per replicate, expressed as a proportion of the total number of species across two replicates, is directly related to the similarity between the replicates. Low JC implies that each replicate contains a relatively low proportion of the species across the pair of replicates; high JC implies that each replicate contains a high proportion of the species in the two replicates.

Table 1. Summary of fish data collected from 37 river sites including seven revisits (a) in Oregon in 1997–1998.

Site	TSR_{obs}	TSR_{tru}	TNI	Site	TSR_{obs}	TSR_{tru}	TNI
S002	12	13.3	190	S458a	9	10.8	242
S028	8	8.9	576	S460	10	11.2	70
S070	7	7.3	198	S009	14	20.8	356
S179	9	11.8	305	S027	9	9.9	180
S208	2	2.0	70	S029	11	14.4	175
S208a	3	3.7	75	S029a	10	11.0	174
S215	12	12.0	224	S067	8	9.1	186
S216	8	8.6	632	S091	7	8.5	329
S250	12	15.2	318	S091a	5	5.6	236
S311	3	3.1	203	S113	8	13.4	163
S420	6	6.7	161	S117	8	10.0	260
S430	15	18.0	162	S133	10	11.5	246
S444	10	10.5	155	S135	10	11.7	268
S447	9	11.3	189	S175	12	15.1	334
S449	11	12.4	173	S179a	11	13.3	258
S449a	16	19.1	383	S179a	9	10.4	212
S450	11	16.0	153	S181	16	19.8	344
S450a	12	15.9	362	S191	6	6.6	242
S458	11	14.0	175				
Maximum					16	20.8	632
Minimum					2	2.0	70
Average					9.46	11.43	242.68
SD					3.29	4.43	118.52

Note: TSR_{obs}, total number of species observed; TSR_{tru}, total species richness estimated from eq. 9; TNI, total number of individuals.

Relating TSR_{tru} to JC

Suppose that we repeatedly drew pairs of replicates from a pool of species and that for each pair of replicates, JC = 1. In this case, each replicate contains the complete species pool; $TSR_{obs} = TSR_{tru}$ and $\%TSR_{obs}$ is an unbiased estimator of $\%TSR_{tru}$. Now suppose that for each pair of replicates, JC = 0. In this case, each replicate is taxonomically unique; TSR_{tru} is far greater than TSR_{obs} and average $\%TSR_{tru}$ per replicate theoretically approaches 0. However, eq. 6 indicates that $\%TSR_{obs} = 50$ when JC = 0, i.e., $\%TSR_{obs}$ as an estimate of $\%TSR_{tru}$ is biased high. This apparent positive bias in $\%TSR_{obs}$ as a potential approximation of TSR_{tru} might be corrected by relating the bias to JC. Except in the extremes, it is not clear yet how the bias is quantitatively related to JC. We will initially assume a linear relationship and then examine both the magnitude and the linearity of the bias defined as $\%TSR_{obs} - \%TSR_{tru}$ later:

$$\text{Bias} = 50(1 - \text{JC}) \tag{7}$$

$\%TSR_{tru}$ can now be related to $\%TSR_{obs}$ by correcting for the bias as follows:

$$\%\text{TRS}_{\text{tru}} = \%\text{TRS}_{\text{obs}} - 50(1 - \text{JC})$$

Replacing $\%TSR_{obs}$ with eq. 6 yields

$$\%\text{TRS}_{\text{tru}} = (50 + 50\text{JC}) - 50(1 - \text{JC}) = 100\text{JC} \tag{8}$$

From eq. 8, TSR_{tru} can be estimated immediately as follows:

$$\text{TRS}_{\text{tru}} = \frac{\overline{\text{SR}}}{\text{JC}} \tag{9}$$

Equation 8 predicts a 1:1 relationship between the average $\%TSR_{tru}$ per replicate and JC between the two replicates. The important implication of eq. 9 is that any two replicates (0.5*N* units each) drawn from total *N* units contain sufficient information with which to estimate the total number of species (TSR_{tru}). The magnitude of the bias (expressed in eq. 7) in the relationship is a critical element in the degree to which eqs. 8 and 9 are valid. We examined the bias as well as eqs. 8 and 9 themselves using data from four fish surveys.

Data sets

We obtained two unpublished and two published fish data sets collected from Oregon (OR), Virginia (VA), and Wyoming (WY) streams and rivers to test the new approach and to estimate sampling effort for each site to reach specified levels of $\%TSR_{tru}$.

Oregon rivers

Forty-one river sites on 26 rivers randomly chosen in OR were surveyed in 1997 and 1998 using one-pass electrofishing. Approximately 90% of the rivers were Strahler fourth to seventh order on 1 : 100 000 scale maps. Eleven sites were visited twice and represent temporal replicates. Each site was divided into 10 transects (sample units), each of which was equal to 10 wetted channel widths (CWs). Widths were measured with a laser rangefinder at the upstream ends of these sites. Fewer than 10 transects were sampled at some sites because of access restrictions, safety considerations, or inaccurate estimates of width variability. We excluded all those sites in the analysis. Consequently, a subset of 37 sites (including the temporal replicates) was analyzed (Table 1).

A Smith–Root model 2.5 GPP electrofisher was used, set at pulsed direct current, 30–60 pulses·s^{-1}, low–high range, and 400–1000 V as needed to roll fish but minimize injury. Electrofisher clock times typically ranged from 1 to 2 h, which translated into 6–8 h of actual sampling time. Fish were collected along the entire transect and all individuals were identified and counted. Species abundance was recorded separately for each transect. The total

Table 2. Summary of fish data collected from 10 Oregon streams in the Cascade Mountains (C) and the Willamette Valley (V) in 1992.

Stream	Total habitat units	Total CWs	TSR_{obs}	TSR_{tru}	Average SR per unit	TNI
C1	15	44	8	8.5	5.93	2558
C2	14	94	4	4.7	2.57	786
C4	18	77	5	5.6	3.67	627
C5	23	78	9	9.2	6.30	2580
V1	8	69	7	7.7	4.88	921
V2	15	73	9	9.7	4.87	852
V3	11	61	11	15.9	4.00	889
V4	26	73	10	11.4	5.42	1541
V5	17	81	5	5.2	2.65	274
V6	19	55	6	7.2	2.89	1693
Maximum	26	94	11	15.9	6.3	2580
Minimum	8	44	4	4.7	2.6	274
Average	16.6	70.5	7.4	8.5	4.3	1272.1
SD	5.32	14.18	2.37	3.35	1.4	796.43

Note: TSR_{obs}, total number of species observed; TSR_{tru}, total species richness estimated from eq. 9; TNI, total number of individuals; SR, species richness in a sample unit.

Table 3. Summary of fish data collected from three Virginia streams (from Angermeier and Smogor 1995).

Copper Creek			Beaver Creek			Bernards Creek		
Habitat unit	SR	TNI	Habitat unit	SR	TNI	Habitat unit	SR	TNI
1	13	579	1	23	349	1	5	25
2	20	581	2	25	215	2	10	25
3	19	791	3	16	274	3	10	48
4	10	172	4	16	207	4	5	13
5	17	1534	5	12	179	5	8	45
6	19	2567	6	14	56	6	5	15
7	20	1561	7	7	29	7	9	29
8	12	153	8	16	187	8	16	89
9	16	1200	9	20	161	9	7	29
10	20	1818	10	14	142	10	10	45
11	18	1232	11	23	267	11	5	9
12	20	1360	12	16	76	12	9	49
13	19	1943	13	9	80	13	5	16
14	20	1558	14	16	144	14	9	29
15	12	157	15	20	184	15	6	39
			16	24	211	16	6	23
			17	18	133	17	5	11
			18	20	182			
Total	24	17 206		31	3076		22	539
TSR_{tru}	25.1			32.4			25.4	
Maximum	20	2567		25	349		16	89
Minimum	10	153		7	29		5	9
Average	17	1147.1		17.2	170.2		7.6	31.7
SD	3.5	723.8		5.0	80.7		3.0	19.8

Note: SR, species richness in a sample unit; TNI, total number of individuals; TSR_{tru}, total species richness estimated from eq. 9.

number of species recorded at each site ranged from two to 16 and the number of individuals ranged from 70 to 632 (see Table 1).

Oregon streams

Sixteen stream sites in two OR ecoregions (the Willamette Valley and the Cascade Mountains) were surveyed with one-pass electrofishing in 1992. Six streams were excluded from this study because either only one species was recorded in the entire site (and also in almost every sample unit) or too few habitat units were sampled. The CW was less than 8 m in all retained streams except at one.

A habitat unit (pool or riffle) was taken as the sample unit in this survey. Each sample unit was sampled in an upstream direction. The upstream and downstream ends were blocked with nets before sampling. The number of sample units ranged from eight to 26, corresponding to 44–98 CWs. A Smith–Root model 12 POW DC electrofisher was used with 700–1000 V. Electrofisher clock time varied among the streams and sampling time ranged from less than 1 to 14 h. All individuals collected were identified and

Table 4. Summary of fish data collected from nine Wyoming streams (Patton et al. 2000).

Stream	Total habitat units	Total CWs	TSR_{obs}	TSR_{tru}	Average SR per unit	TNI
Site 1	16	222	7	7.0	6.3	5493
Site 2	16	118	11	11.0	8.9	7404
Site 3	16	98	13	13.0	8.8	1721
Site 4	16	320	9	9.0	6.5	1699
Site 5	16	111	14	14.0	10.3	4394
Site 6	16	216	7	7.0	4.1	969
Site 7	16	276	8	8.0	6.1	4894
Site 8	16	78	10	10.1	7.6	1215
Site 9	16	140	10	10.0	6.4	529
Maximum		320	14	14.0	10.3	7404
Minimum		78	7	7.0	4.1	529
Average		175.4	9.9	9.9	7.2	3146.4
SD		85.9	2.5	2.5	1.9	2441.6

Note: TSR_{obs}, total number of species observed; TSR_{tru}, total species richness estimated from eq. 9; TNI, total number of individuals.

counted. The data from each habitat unit were recorded separately. The total number of species recorded at each site varied from four to 11, with total numbers of fish counted ranging from 274 to 2580. The data set is summarized in Table 2.

Virginia streams

Angermeier and Smogor (1995) reported a fish survey on three VA streams: Copper Creek, Beaver Creek, and Bernards Creek. They used habitat units (pools, riffles, or runs) as sample units and conducted two-pass electrofishing. The number of individuals of each species was recorded for each habitat unit. The numbers of habitat units surveyed differed among the three streams, 15 in Copper Creek, 18 in Beaver Creek, and 17 in Bernards Creek, which corresponds to 61, 67, and 81 CWs, respectively. A total of 24, 31, and 22 species (including a hybrid) were recorded in the three streams, respectively. The total number of individuals collected was 17 206 at Copper Creek, 3076 at Beaver Creek, and 539 at Bernards Creek (Table 3). More details regarding these three streams can be found in Angermeier and Smogor (1995).

Wyoming streams

Patton et al. (2000) surveyed nine stream sites in the Missouri River drainage of eastern WY. The CWs of these streams ranged from 2.5 to 10.2 m. They delineated 16 contiguous 50-m-long reaches at each site. Electrofishing and seining were used alternatively in the 16 sample units. The number of individuals for each species was recorded at each sample unit. In a two-dimensional plot of detrended correspondence analysis, the units sampled using the two sampling methods at each site were highly similar compared with the differences among sites. Consequently, we considered each 50-m reach as a sample unit regardless of sampling method, yielding 144 sample units across all nine sites. The species richness recorded at these sites ranged from seven to 14. The total number of individuals collected at a site also varied substantially, from 529 at Site 9 to 7404 at Site 2 (Table 4).

Analysis methods

Species accumulation curves

We used randomization procedures for resampling to simulate samples of different sizes for constructing species accumulation curves and determining average JC for each sample size. For the species accumulation curves, samples of one, two, three, four, etc., sample units were drawn at random from the pool of sample units (without replacement). The richness was calculated for each draw and then averaged across 1000 random draws and plotted against sample size. For example, to estimate average richness for a sample size of three sample units, 1000 random draws of three sample units were taken from the pool of sample units and richness determined for each draw and then averaged across the 1000 random draws.

Calculation of average JC

For the JC – sample size relationships, samples of two, four, six, etc., sample units were drawn at random from the pool of sample units (without replacement). Each draw was split evenly and then JC calculated between the splits. The process was repeated 1000 times and JC for each sample size averaged across the 1000 draws and plotted against sample size. After examining species curves for all sites in the four data sets, we identified 13 sites where species curves entirely leveled and one site where the species curve stabilized, i.e., TSR_{tru} appears to be reached or approached. We used these 14 sites to test eqs. 7–9. We then estimated the sampling effort needed in these three surveys to achieve fixed proportions of total richness.

Fitting the $\%TSR_{tru}$–JC relationship

In order to validate eqs. 7–9, we need to know TSR_{tru} at a site. As shown later, at 14 sites across the four data sets, species curves leveled or stabilized, meaning that TSR_{tru} was likely reached or approached. These 14 sites were chosen to examine the $\%TSR_{tru}$–JC relationship. First, we calculated $\%TSR_{tru}$ for a series of sample sizes (1, 2, 3,..., 0.5N units pooled) as $100 \times (\overline{SR}/TSR_{tru})$. Then, we fitted $\%TSR_{tru}$–JC curves with an appropriate model and compared the fitted model with eq. 8.

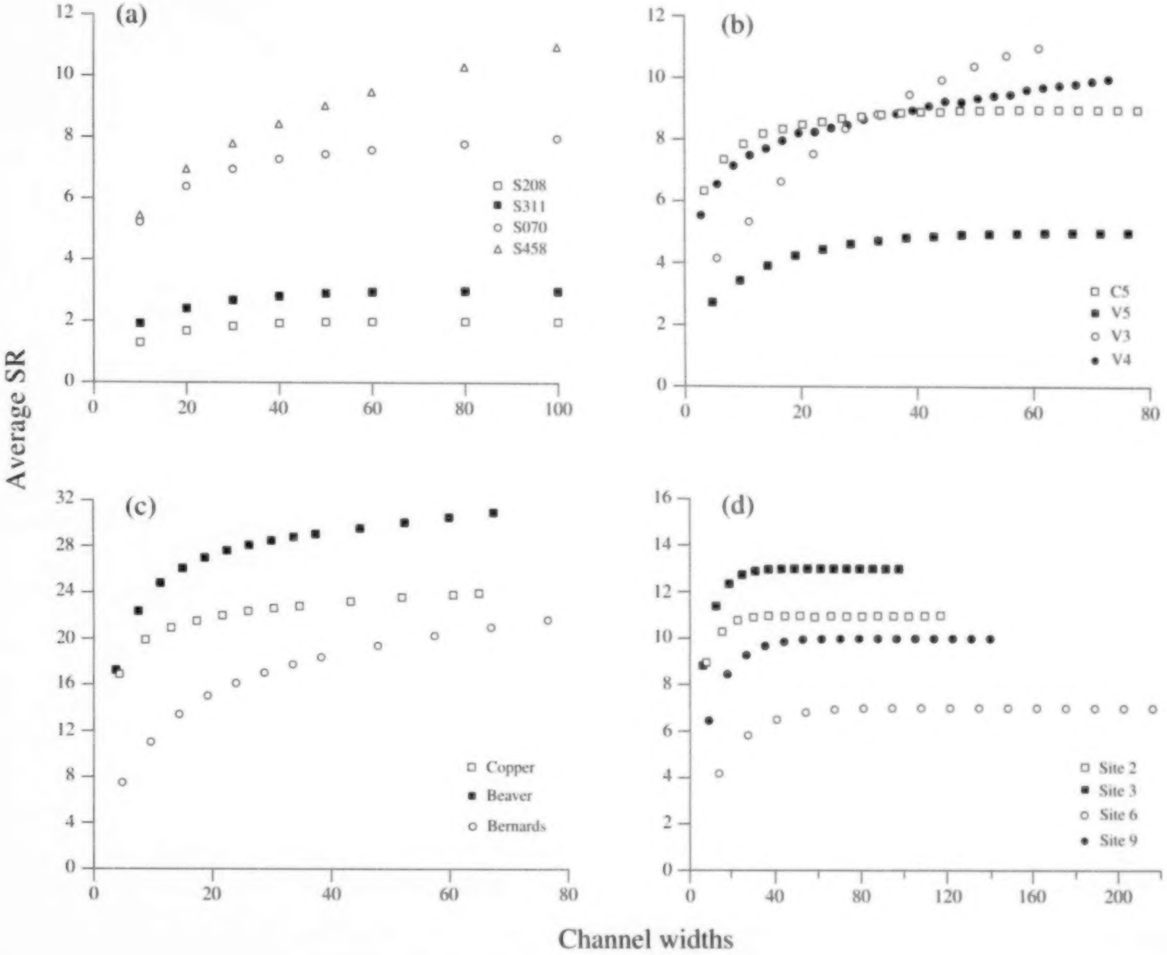
We also tested eq. 7 for a bias–JC relationship. First, we randomly selected 2n sample units from the pool of sample units at each site (n = 1, 2, 3,..., 0.5N) and set the total richness across the 2n sample units as TSR_{obs}. We calculated $\overline{SR}$ per replicate (n units) and divided it by TSR_{obs} to get $\%TSR_{obs}$ and by TSR_{tru} to get $\%TSR_{tru}$. JC was also calculated for each pair of replicates. This process was repeated 1000 times and yielded an average for each sample size.

Results

Species accumulation curves

Species accumulation curves varied significantly among different sites of all four data sets (Fig. 1). At most OR and VA sites, species curves did not level but kept increasing to varying extents. Of the 37 OR river sites, the species curves entirely leveled at only two sites, S208 and S311, where two

Fig. 1. Species accumulation curves vary substantially among different sites across all four data sets: examples for (*a*) OR rivers, (*b*) OR streams, (*c*) VA streams, and (*d*) WY streams. This suggests that different sampling efforts are required for reaching TSR_{tru} at different sites. The species curves level entirely at four OR sites (S208, S311, C5, and V5) and all WY sites and the species curve stabilizes at Copper Creek, implying that TSR_{tru} was reached or approached at these sites. Species richness here is the average species richness across 1000 simulated samples.



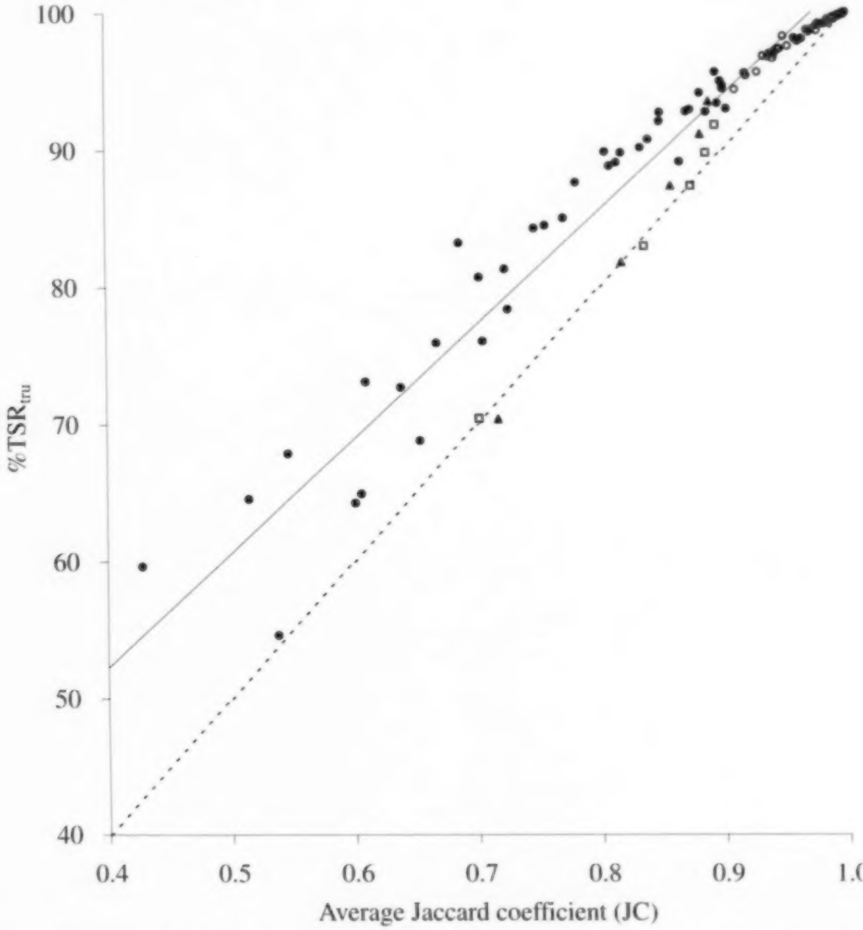
and three species were recorded, respectively (Fig. 1*a*). At the 10 OR stream sites, species curves entirely leveled at C5 and V5, where five and nine species were recorded (Fig. 1*b*). Of the three VA creeks, the species curve stabilized at Copper Creek (Fig. 1*c*). The WY data set is extraordinary, where species curves leveled at all sites (Fig. 1*d*). When species curves leveled or stabilized, TSR_{tru} was assumed to be reached or approached. In addition, the high variation in species curves among sites suggests that TSR_{tru} or the same proportion of TSR_{tru} would require very different sampling efforts at different sites.

$\%TSR_{tru}$–JC relationship and the effect of sample size on TSR_{tru} estimations

The 13 sites where species curves completely leveled were chosen to test eq. 8. The TSR_{tru} at these sites ranged from 2 to 14. To broaden the scope of species richness in this test, we included Copper Creek in VA, where 24 species were recorded and the species curve stabilized.

$\%TSR_{tru}$ was plotted against average JC (Fig. 2), together with $\%TSR_{tru}$ predicted from eq. 8, for each sample size at each of the 14 sites. Although we used a linear fit to the estimates of bias, the form of the relationship can be nonlinear, as shown at Copper Creek and C5 in Fig. 2. Equation 8 fit some sites well (e.g., C5 and Copper Creek) but underestimated $\%TSR_{tru}$ at other sites, but the difference between observed and estimated $\%TSR_{tru}$ was usually small. On average, the deviation from 100JC is 3.5% TSR_{tru} with a standard error of 3.6%. The underestimation tended to increase at lower levels of JC at several sites. For example, JC reached 0.43 at the one sample unit level at WY Site 6, and the deviation reached 16.5%. In spite of the variation among the sites and over the ranges of sample size, the overall correlation was high (Pearson's $r = 0.97$) and a linear regression

Fig. 2. Positive and strong correlation between $\%\mathrm{TSR}_{\mathrm{tru}}$ and autosimilarity (JC) over the ranges of JC available ($r = 0.97$) or for JC 0.9 only (i.e., open circles were excluded) ($r = 0.95$) at the 14 test sites at which $\mathrm{TSR}_{\mathrm{tru}}$ was reached or approached. The relationship is fitted with a linear regression (continuous line) for JC 0.9 compared with the prediction from 100JC (broken line). Copper Creek (□) and C5 (▲) fit 100JC better than the others.



fitted the relationship adequately ($r^2 = 0.94$): $\%\mathrm{TSR}_{\mathrm{tru}} = 22.44 + 78.46 \times \mathrm{JC}$. When $\mathrm{JC} > 0.9$, $\%\mathrm{TSR}_{\mathrm{tru}}$ is 98.7% on average and >95% in all cases. For $\mathrm{JC} \leq 0.9$, the regression is even closer to 100JC than for the whole range: $\%\mathrm{TSR}_{\mathrm{tru}} = 18.9 + 83.4 \times \mathrm{JC}$ (Fig. 2).

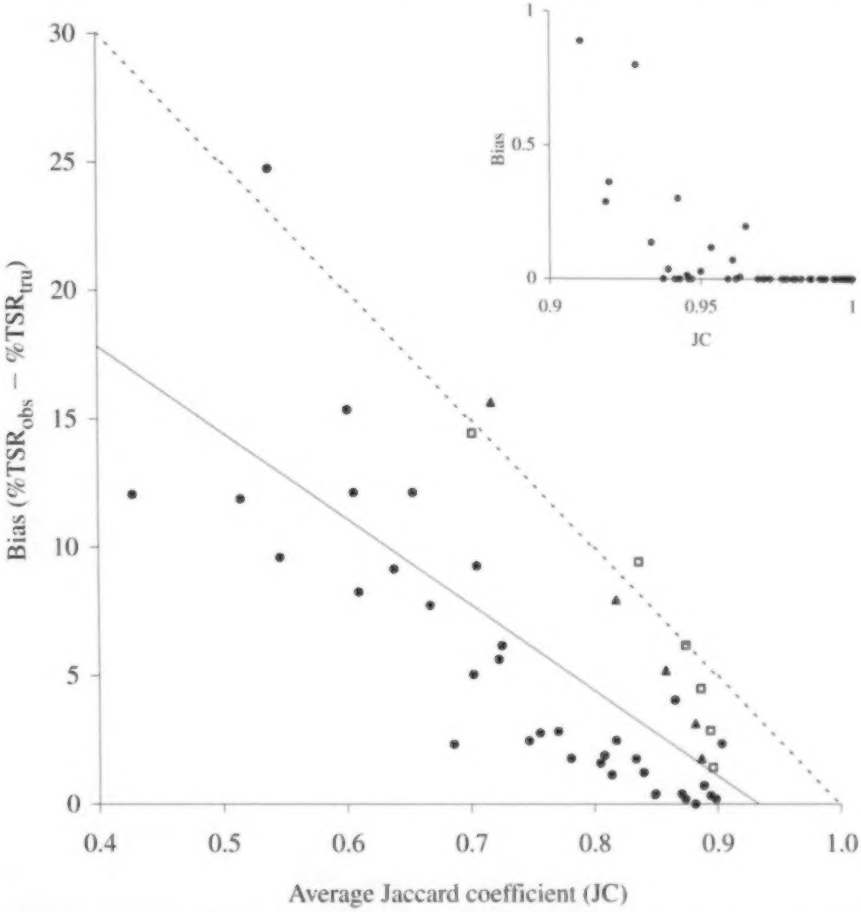
The bias in $\%\mathrm{TSR}_{\mathrm{obs}}$ was plotted against average JC in two parts in Fig. 3: for the range of $\mathrm{JC} \leq 0.90$ and $\mathrm{JC} > 0.90$ as an inset shown in the figure. Equation 7 better predicted the bias in $\%\mathrm{TSR}_{\mathrm{obs}}$ at some sites such as C5 and Copper Creek than at others. But a linear regression across all sites fit the bias–JC relationship adequately ($r^2 = 0.56$): bias = $31.8 - 33.4 \times \mathrm{JC}$ (for $\mathrm{JC} \leq 0.90$). When both bias estimators were applied to the 37 OR river sites, they yielded similar results with only 6.4% $\mathrm{TSR}_{\mathrm{tru}}$ difference on average. This suggests that the deviation from eq. 8 may not present a major problem in practice. For $\mathrm{JC} > 0.90$, the bias was always <1% and consequently a correction (eq. 7) was unnecessary.

The general approach was further evaluated by comparing predicted $\mathrm{TSR}_{\mathrm{tru}}$ with observed $\mathrm{TSR}_{\mathrm{tru}}$ for the 14 sites and whether the predictions from eq. 9 varied as a function of sample size. In reality, $\mathrm{TSR}_{\mathrm{tru}}$ is always an integer; however, when estimating richness, noninteger values can occur. Trimming predicted $\mathrm{TSR}_{\mathrm{tru}}$ to the nearest integer may have little influence when richness is high but might be quite influential when richness is low. As a result, we used the noninteger estimates to assess predictive capability and the effect of sample size. The predictions of $\mathrm{TSR}_{\mathrm{tru}}$ were accurate at all OR and VA and most WY test sites at different sample sizes (Fig. 4*a*). But overestimations were evident, particularly at WY Sites 3, 5, and 6 for sample sizes of two or less sample units pooled (Fig. 4*b*). These overestimates declined as sample size increased, reflecting the nonlinearity of the bias. However, at sample sizes of greater than three units pooled, predicted and observed $\mathrm{TSR}_{\mathrm{tru}}$ were close regardless of whether we consider the bias linear or nonlinear. This result agrees with Fig. 3 in that eq. 7 could overestimate the bias in $\%\mathrm{TSR}_{\mathrm{obs}}$ at some sites when JC is low.

Estimation of sample size to achieve a standard $\%\mathrm{TSR}_{\mathrm{tru}}$

Equation 9, in combination with the species accumulation

Fig. 3. Negative and strong correlation of the bias in %TSR_{obs} with average JC ($r = -0.72$ for JC 0.9) at the 14 test sites where TSR_{tru} is known, showing that eq. 7 (broken line) tended to overestimate the bias (solid line, linear fit) at low JC levels, and also, the bias is minor and can be ignored when JC > 0.90 (see inset). Copper Creek (□) and C5 (▲) fit eq. 7 better than the others.



curves (Fig. 1), can be used to estimate the sample size needed to reach any particular %TSR_{tru} or a proportion of the local species pool. The species curves in all four data sets could be fit with a log function: SR = $a + b \times$ log(sample size) with r^2 typically >95%. Substituting TSR_{tru} or a proportion of TSR_{tru} for SR in the log function allowed us to estimate the needed sample sizes, summarized in Table 5, for 90, 95, and 100% TSR_{tru}.

An average of 286 CWs are required for 100% TSR_{tru} for the OR river sites, with high variability (70–1383 CWs). The sampling effort for 90% TSR_{tru} required about half (36–762 CWs). At the OR stream sites, 100% TSR_{tru} required 67–457 CWs, 198 on average. The three VA streams required smaller sampling effort for the same %TSR_{tru}, ranging from 82 to 134 CWs for 100% TSR_{tru}. The WY sites required the least sampling effort for the same %TSR_{tru}, 14–121 CWs for 100% TSR_{tru}.

We also estimated the %TSR_{tru} reached by a standard sample size of 100 CWs in OR river sites. The %TSR_{tru} reached by this sample size ranged from 60 to 99%, with an average of 84% across all 37 sites. The %TSR_{tru} reached by 100 CWs was negatively and significantly correlated with the estimated or observed TSR_{tru} (Pearson's $r = -0.43$, $P < 0.01$). However, the relatively low r value suggests that other factors, such as species abundance structure, may also have influenced the %TSR_{tru}. The sample size required for 100% TSR_{tru} at the OR and WY stream sites was also positively correlated with TSR_{tru} ($r = 0.45$ and 0.40, respectively) but not significantly at the 5% level. In the three VA streams, Bernards Creek required the largest sample size for the same level of %TSR_{tru}, while the highest richness was recorded at Beaver Creek.

Discussion

When survey data are used to detect and describe spatial–temporal patterns of species diversity and assemblage structure, it is desirable to know what proportion of all species in a local species pool is reached with the sampling effort used. However, this question cannot be answered directly if species curves do not level and TSR_{tru} is unknown. A standard sample size used in routine surveys may well attain different proportions of the species pool across different sites, intro-

Fig. 4. Estimations of TSR_{tru} using the JC-based approach (eq. 9) are accurate, being little affected by sample size (the number of sample units pooled) at five OR and VA test sites (*a*), but overestimations are evident at most WY sites when sample sizes are small (*b*). The broken lines indicate real TSR_{tru} at each site. TSR_{tru} of Copper Cr. is re-scaled by multiplying by 0.5 in the figure.

ducing biases into both species richness and composition comparisons (Cao et al. in press).

The approach that we propose in this study can be used to easily and reliably estimate $\%TSR_{tru}$ reached by a given sampling effort at a site. Equation 8 is a straightforward, quantitative relationship between $\%TSR_{tru}$ and autosimilarity (JC). Autosimilarity is ecologically meaningful and explicitly defined with a fixed range (0–1). The 1:1 ratio between $\%TSR_{tru}$ and JC in eq. 8 and the usefulness of eq. 9 for estimating TSR_{tru} were strongly supported by the field data. A large number of approaches have been proposed for estimating TSR_{tru} (Colwell and Coddington 1994; Hellmann and Fowler 1999). A major weakness of these approaches is that estimated TSR_{tru} is affected by sample size (Smith and Van Belle 1984; Hellmann and Fowler 1999; Lande et al. 2000). One of these approaches, the Michaelis–Menten model, used in previous studies on sampling sufficiency for fish surveys (Lyons 1992; Angermeier and Smogor 1995) has been criticized recently on theoretical and practical grounds (e.g., Palmer 1990; Keating and Quinn 1998). Our results showed that the new approach predicted TSR_{tru} accurately for the sites tested and was little affected by sample size. More important is that JC is a direct measure of $\%TSR_{tru}$ and can therefore be easily used to determine when sampling is sufficient in the field so that the problem of over- or under-sampling with a standard sample size could be overcome. Other estimators of TSR_{tru} typically involve more complex calculations in determining what $\%TSR_{tru}$ is reached.

Several technical issues may cause concern regarding the new approach and the underlying assumptions. First is the choice of similarity measure. The present study chose a binary index, the JC, for several reasons: (*i*) the $\%TSR_{tru}$ used to measure sampling sufficiency is related to species presence–absence only, (*ii*) JC is easy to calculate and widely accepted, and (*iii*) JC can be strictly and explicitly related to $\%TSR_{tru}$, as we have shown in this study. Other similarity indices (such as the Sørenson index and Bray–Curtis index) do not appear to have all of these advantages. Second, eq. 8 assumes a linear relationship between JC and $\%TSR_{tru}$ across their full range. Both JC and $\%TSR_{tru}$ examined in the present study fall into their upper ranges because of the relatively low species richness at these sites. It is not clear whether the linear relationship holds for the lower range. However, the $\%TSR_{tru}$–JC relationship over the lower range is less important because a low JC and $\%TSR_{tru}$ implies that a low proportion of the species pool is sampled and sampling is insufficient. Third, the prediction of $\%TSR_{tru}$ from JC might be influenced by highly left-skewed species abundance distributions. For example, when there are a few very common species and many rare species in a sample, as in S009 and S113A in the OR river data set, a relatively high JC can be reached at small sample sizes, implying a high $\%TSR_{tru}$. Under these circumstances, as sample size increases, more rare species are included and JC tends to decrease, implying a low $\%TSR_{tru}$, although JC eventually increases further. A large sample should be taken at such sites to get a reasonable estimation of JC and $\%TSR_{tru}$. A further question is the variation of catchability among species, and catchability is also affected by sampling methods, habitat conditions, and fish sizes (Bayley and Peterson 2001). Bayley and Peterson (2001) developed an empirical Bayesian model to correct this bias in estimating species richness in a sample unit. For estimating site/local species pools as in the present study, multiple sample units are often required and a large number of individual fish are collected. Variable catchability thus appears unlikely to present a major problem.

Several studies have focused on sampling effort in stream fish surveys (e.g., Lyons 1992; Angermeier and Smogor 1995; Patton et al. 2000), but no reports are available for rivers. The numbers of CWs estimated for the same $\%TSR_{tru}$ in the OR rivers in this study are generally higher than those estimated for the OR streams and others. The present study estimated 70–1383 CWs for 100% TSR_{tru} for the OR rivers. In comparison, 67–457 CWs are required for 100% TSR_{tru} at the 10 OR stream sites in the present study, 35 CWs reached ≥95% TSR_{tru} at 10 stream sites in Wisconsin (Lyons 1992), 35–158 CWs could capture all species in 25 stream sites in South Carolina (Paller 1995), 43–92 CWs are required for 95% TSR_{tru} at three VA streams (22–67 CWs in Angermeier and Smogor 1995), and 14–121 CWs captured all species in nine WY sites (14–50 CWs in Patton et al. 2000). The large difference in sampling effort required between rivers and streams is probably related to different species capture efficiencies. Stream surveys usually cover the whole channel and all habitats can be sampled. However, fish sampling in rivers normally only covers part of the channel and the proportion of the sampled channel covered is relatively low. For example, species inhabiting the bottom of deep pools (i.e., white sturgeon) are not likely to be collected by electrofishing. Thus, the numbers of CWs, as an expression of sampling effort, are not quite comparable between streams and rivers. Rivers also usually support more diverse fish assemblages, which likely require higher sampling effort to characterize (see below).

Sampling effort required is also related to sampling methods and data analytical approaches. Two-pass (versus one-pass) electrofishing should at least partially explain why the three VA streams required lower sampling effort than the OR streams. For the WY data, we did not differentiate the sample units collected using electrofishing and seining. This may account for the difference between our estimation and the result of Patton et al. (2000).

However, why does sampling effort required for the same $\%TSR_{tru}$ vary greatly among the streams and rivers of similar sizes when the same sampling techniques, effort, and estimation method are used? There appear to be multiple reasons, but from the standpoint of data analysis, two factors are critical. First, as shown by the OR rivers data, sampling sufficiency was negatively and significantly correlated with TSR_{tru}, which implies that larger samples are required to characterize more diverse assemblages. The general correla-

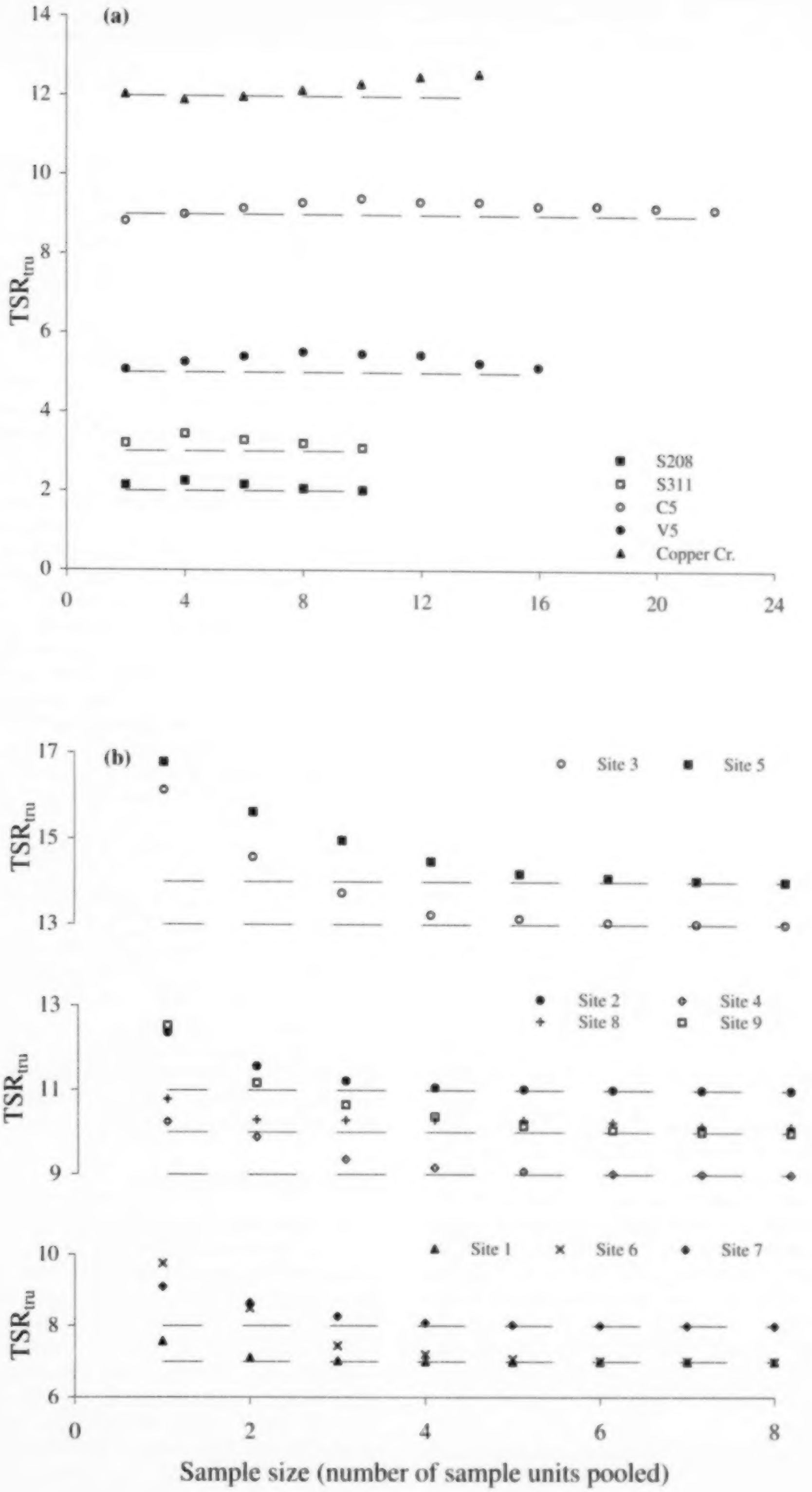

(a)
TSR$_{tru}$
14
12
10
8
6
4
2
0
0
4
8
12
16
20
24
S208
S311
C5
V5
Copper Cr.
(b)
Site 3
Site 5
TSR$_{tru}$
17
15
13
Site 2
Site 4
Site 8
Site 9
TSR$_{tru}$
13
11
9
Site 1
Site 6
Site 7
TSR$_{tru}$
10
8
6
0
2
4
6
8
Sample size (number of sample units pooled)

Table 5. Estimated numbers of CWs required for different $\%TSR_{tru}$ in three data sets.

		Data set			
Sampling sufficiency	Sampling effort	OR rivers (N = 37)	OR streams (N = 10)	VA streams (N = 3)	WY streams (N = 9)
100% TSR_{tru}	Maximum	1383	457	134	121
	Minimum	70	67	82	14
	Mean	286	198	103	41
	SD	251	138	28	33
95% TSR_{tru}	Maximum	1027	288	92	64
	Minimum	50	36	43	6
	Mean	214	126	63	25
	SD	184	82	26	18
90% TSR_{tru}	Maximum	762	182	48	33
	Minimum	36	20	33	3
	Mean	161	82	40	15
	SD	136	52	10	10

Note: TSR_{tru}, total species richness estimated from eq. 9.

tion between species richness and habitat heterogeneity has been well established (e.g., Douglas and Lake 1994; Ricklefs and Lovette 1999; Therriault and Kolasa 1999). Thus, more heterogeneous habitats tend to support more diverse fish assemblages and likely require more sampling effort to be characterized adequately. However, this relationship can be easily compounded by another factor, i.e., species abundance distribution. A highly left-skewed species abundance curve means that new species are added slowly but continuously with increasing sample size. For example, 36% of the species at Bernards Creek occurred in <20% of the habitat units, while only 16% of the species at Beaver Creek fell into this category. Consequently, a larger sample size was required to achieve the same level of $\%TSR_{tru}$ at Bernards Creek than at species-richer Beaver Creek. Similarly, more species in the OR streams are at low frequencies than in the WY streams. As a result, the OR streams require more sampling effort than the WY streams for the same level of $\%TSR_{tru}$. It is not clear whether and how species abundance structure is related to habitat heterogeneity or habitat types, so it is difficult to prejudge the sampling effort based on habitat characteristics. Autosimilarity (JC) indicates the overall level of biological and habitat heterogeneity and provides a solid basis for determining sampling effort.

The present study covers three markedly different geographic regions and a wide range of stream and river sizes, and the species richness examined is quite typical for North American streams and rivers. However, it is not clear how well eqs. 8 and 9 work for more diverse taxonomic assemblages, such as macroinvertebrates and algae, or predict the %TSR–JC relationship at large spatial scales for fish and other taxa. A need for further evaluation is warranted. Simulation experiments could be highly useful for such evaluations. At the present time, we would suggest restricting the application of the new approach to fish assemblages at local scales. How it can be applied to fish surveys in the field is described below.

JC is easy to calculate and the majority of fish species can be identified or differentiated in the field by trained crews. This makes it possible to determine sampling sufficiency on site. When N sample units are surveyed, the data from each unit can be recorded separately. Two options are available to evaluate sampling sufficiency. One relies on the use of resampling procedures. The species data can be entered into a portable computer, by sample unit, and the resampling process run to estimate an average JC at the sample size of $0.5N$. A second option is to assign the N sample units to two groups ($0.5N$ per group) using a table of random numbers ($1 - N$). The species data for the first group are pooled to create one replicate, while the other group forms the second replicate. JC can be easily calculated for these two replicates. In either case, if the JC does not reach a desired level, two more units should be sampled and either of the two options rerun. This process can be repeated until the desired JC level is reached, a process similar to sequential sampling (Jackson and Resh 1988). The JC level estimated using the second option is based on one combination only, providing an approximation of average JC. It should be refined at the data analysis stage using the resampling techniques. The relative ease of use of eqs. 8 and 9 allows considerable flexibility in guiding sampling effort. For some purposes, estimating TSR_{tru} might be sufficient, in which case a sampling effort achieving a JC in the range of 0.5–0.6 may be sufficient. For other purposes, such as obtaining most of the species present for community comparisons, achieving a higher level, such as 0.8–0.9, might be desirable.

Acknowledgements

We thank Paul Angermeier, Wayne Hubert, John Lyons, and two anonymous reviewers for their constructive comments on an earlier version of this manuscript. Paul and Tim Patton kindly provided us their data, which considerably broadened the scope of our analysis. Discussions with John Van Sickle and Scott Urquhart clarified several statistical questions. Alan Herlihy assisted in preparing the data set of OR streams. This research was funded by the NRC Associateship Program through EPA cooperative agreement CR826388 and EPA contract 68-C6-0005 to Dynamac. This manuscript has been subjected to the Agency's peer and administrative review, and it has been approved for publication.

Mention of trade names or commercial products does not constitute endorsement or recommendation for use.

References

Allan, J.D., and Flecker, A.S. 1993. Biodiversity conservation in running waters. BioScience, **43**: 32–43.

Angermeier, P.L., and Smogor, R.A. 1995. Estimating number of species and relative abundances in stream-fish communities: effects of sampling effort and discontinuous spatial distributions. Can. J. Fish. Aquat. Sci. **52**: 936–945.

Angermeier, P.L., and Winston, M.R. 1999. Characterizing fish community diversity across Virginia landscapes: prerequisite for conservation. Ecol. Appl. **9**: 335–349.

Barbour, M.T., Gerritsen, J., Snyder, B.D., and Stribling, J. 1999. Rapid bioassessment protocols for use in streams and rivers: periphyton, benthic macroinvertebrates and fish. EPA 841-D-99-002. U.S. Environmental Protection Agency, Washington, D.C.

Bayley, P.B., and Peterson, J.T. 2001. An approach to estimate probability of presence and richness of fish species. Trans. Am. Fish. Soc. **130**: 620–633.

Bayley, P.B., Larimore, R.W., and Dowling, D.C. 1989. Electric seine as a fish-sampling gear in streams. Trans. Am. Fish. Soc. **118**: 447–453.

Benson, B.J., and Magnuson, J.J. 1992. Spatial heterogeneity of littoral fish assemblages in lakes: relation to species diversity and habitat structure. Can. J. Fish. Aquat. Sci. **49**: 1493–1500.

Cao, Y., Williams, D.D., and Larsen, D.P. Comparisons of ecological communities: the problem of sample representativeness. Ecology. In press.

Colwell, R., and Coddington, J.A. 1994. Estimating terrestrial biodiversity through extrapolation. Philos. Trans. R. Soc. Lond. B. Biol. Sci. **345**: 101–118.

Douglas, M., and Lake, P.S. 1994. Species richness of stream stones: an investigation of the mechanisms generating the species–area relationship. Oikos, **69**: 387–396.

Gauch, H.J. 1982. Multivariate analysis in community ecology. Cambridge University Press, Cambridge, U.K.

Hellmann, J.J., and Fowler, G.W. 1999. Bias, precision, and accuracy of four measures of species richness. Ecol. Appl. **9**: 824–834.

Hughes, R.M., Kaufmann, P.R., Herlihy, A.T., Kincaid, T.M., Reynolds, L., and Larsen, D.P. 1998. A process for developing and evaluating indices of fish assemblage integrity. Can. J. Fish. Aquat. Sci. **55**: 1618–1631.

Jackson, D.A., and Harvey, H.H. 1989. Biogeographic associations in fish assemblages: local vs. regional processes. Ecology, **70**: 1472–1484.

Jackson, J.K., and Resh, V.H. 1988. Sequential decision plans in monitoring benthic macro-invertebrates: costs savings, classification accuracy, and development of plans. Can. J. Fish. Aquat. Sci. **45**: 280–286.

Karr, J.R. 1991. Biological integrity: a long-neglected aspect of water resource management. Ecol. Appl. **1**: 66–84.

Keating, K.A., and Quinn, J.F. 1998. Estimating species richness: the Michaelis–Menten model revisited. Oikos, **81**: 411–416.

Kent, M., and Coker, P. 1992. Vegetation description and analysis. CRC Press, Ann Arbor, Mich.

Lande, R., DeVries, P.J., and Walla, T.R. 2000. When species accumulation curves intersect: implications for ranking diversity using small samples. Oikos, **89**: 601–605.

Legendre, P., and Legendre, L. 1998. Numeric ecology. 2nd ed. Elsevier, New York.

Lyons, J. 1992. The length of stream to sample with a towed electrofishing unit when fish species richness is estimated. N. Am. J. Fish. Manag. **12**: 198–203.

MacIntosh, R.P. 1991. Concept and terminology of homogeneity and heterogeneity in ecology. *In* Ecological heterogeneity. *Edited by* J. Kolasa and S.T.A. Pickett. Springer-Verlag, New York. pp. 25–46.

Maret, T.R., Robinson, C.T., and Minshall, G.W. 1997. Fish assemblages and environmental correlates in least-disturbed streams of the Upper Snake River Basin. Trans. Am. Fish. Soc. **126**: 200–216.

Paller, M.H. 1995. Relationships among number of fish species sampled, reach length surveyed, and sampling effort in South Carolina Coastal Plain streams. N. Am. J. Fish. Manag. **15**: 110–120.

Palmer, M.W. 1990. The estimation of species richness by extrapolation. Ecology, **71**: 1195–1198.

Patton, T.M., Hubert, W.A., Rahel, F.J., and Gerow, K.G. 2000. Effort needed to estimate species richness in small streams on the Great Plains in Wyoming. N. Am. J. Fish. Manag. **20**: 394–398.

Ricklefs, R.E., and Lovette, I.J. 1999. The role of island area *per se* and habitat diversity in the species–area relationships of four Lesser Antillean fauna groups. J. Anim. Ecol. **68**: 1142–1160.

Smith, E.P., and Van Belle, G. 1984. Nonparametric estimation of species richness. Biometrics, **41**: 119–129.

Therriault, T.W., and Kolasa, J. 1999. Explicit links among physical stress, habitat heterogeneity and biodiversity. Oikos, **89**: 387–391.

Whittier, T., and Kincaid, T. 1999. Introduced fish in northeastern USA lakes: regional extent, dominance, and effect on native species richness. Trans. Am. Fish. Soc. **128**: 769–783.

Influence of harvest refugia on penaeid prawn population dynamics and sustainable catch

Jason E. Tanner

Abstract: Harvest refuges have frequently been proposed as fishery management tools for species with sedentary adults and dispersive juveniles but less often for species with a highly mobile adult phase. In this paper, I use a population model to investigate the potential of using harvest refuges for managing species with both dispersive juveniles and mobile adults using the penaeid prawn *Penaeus latisulcatus* as a model. Maximum harvest occurred when only a very small proportion of the habitat (2–3%) was fished very heavily ($F = 10$). When only a small percentage of the habitat was trawled, harvest increased as fishing mortality increased, but when trawling was more extensive, harvest peaked with a relatively low fishing mortality. This supports recent contentions that by themselves, marine reserves are insufficient to manage a fishery effectively and that they need to be used in conjunction with effort restrictions. Sensitivity analyses indicated that adult, juvenile, and larval mortality are all near thresholds that qualitatively change model behaviour, but provided their values remain below the threshold, it is not important to know them accurately to determine the best harvest regime.

Résumé : On a souvent proposé l'établissement de refuges contre la pêche comme outil de gestion d'espèces à adultes sédentaires dont les petits se dispersent; mais, on l'a rarement fait dans le cas d'espèces dont les adultes sont très mobiles. On trouvera ici un modèle de population qui permet d'évaluer le potentiel de l'utilisation des refuges dans la gestion d'une espèce dont les petits se dispersent et dont les adultes sont mobiles; la crevette *Penaeus latisulcatus* (Penaeidés) sert d'exemple. Le maximum de captures se produit lorsque la pêche intensive ($F = 10$) est concentrée sur seulement une très petite fraction de l'habitat (2–3%). Lorsqu'on pêche au chalut sur seulement un petit pourcentage de l'habitat, la récolte augmente en même temps que la mortalité due à la pêche, mais lorsque le chalutage se fait sur une surface plus grande, la capture atteint un sommet à des mortalités dues à la pêche relativement basses. Ce résultat vient appuyer des affirmations récentes voulant que les réserves marines en elles-mêmes ne suffisent pas pour une gestion efficace de la pêche, mais qu'elles doivent être utilisées en combinaison avec des restrictions de l'effort de pêche. Des analyses de sensibilité montrent que les mortalités des adultes, des juvéniles et des larves se situent toutes près de seuils qui changent qualitativement le comportement du modèle. Néanmoins, si les mortalités se maintiennent sous les seuils, il n'est pas nécessaire de les connaître de façon précise pour pouvoir déterminer le meilleur régime de récolte.

[Traduit par la Rédaction]

Introduction

Increasing attention is being paid to the use of no-catch areas as a precautionary tool for fishery management. Marine protected areas (MPAs) provide a spatial refuge for exploited stocks, in contrast with traditional management measures (e.g., closed seasons, effort restrictions), which provide a temporal refuge (Bohnsack 1998) and are thus a fundamentally different way of management. However, a number of studies have shown that MPAs by themselves have severe limitations and suggest that they need to be utilised in concert with more conventional effort restrictions (Holland and Brazee 1996; Allison et al. 1998). While protected areas have been a mainstay of terrestrial wildlife management, their use in marine systems is still relatively rare, although increasing, and often meets with fierce resistance from affected fishing industries (e.g., Brailovskaya 1998). It is now widely recognised that MPAs are probably one of the best ways of preserving exploited fish stocks under a wide range of scenarios where it is not possible to gain accurate estimates of the parameters (such as fishing mortality (F) and natural mortality (M)) used in traditional fishery models, restrictions on effort are difficult to enforce, or there is a great deal of unpredictable variability in the dynamics of the exploited populations (e.g., Carr and Reed 1992; Lauck et al. 1998; Sumaila 1998). In these cases, an MPA is often more easily enforced than other catch or effort restrictions and provides a source from which overexploited areas can be recolonised, improving the stock's sustainability (e.g., Buxton 1992; Mangel 1998).

MPAs have been particularly emphasised for the management of relatively sedentary invertebrates, such as abalone and sea urchins (e.g., Dugan and Davis 1993). Many of these species rely on high-density aggregations of adults for successful fertilisation of externally released eggs (Pennington 1985) and are thus vulnerable to even moderate reductions in population size. The use of MPAs to manage species with more mobile adults has received somewhat less atten-

Received September 13, 2000. Accepted July 12, 2001. Published on the NRC Research Press Web site at http://cjfas.nrc.ca on August 22, 2001.
J15968

J.E. Tanner. SARDI Aquatic Sciences, P.O. Box 120, Henley Beach, SA 5022, Australia
(e-mail: tanner.jason@saugov.sa.gov.au).

Can. J. Fish. Aquat. Sci. **58**: 1794–1804 (2001) DOI: 10.1139/cjfas-58-9-1794

tion, although the idea has been around for some time (e.g., Beverton and Holt 1957). For these species, the protection of spawning sites has been emphasised (e.g., Die and Watson 1992; Horwood et al. 1998), and the influence of protecting other areas of the habitat is not as well known. It is generally considered that because adults are likely to move out of protected areas over fairly short time scales, MPAs will be of little use in preventing overexploitation. However, some simulation studies indicate that providing intermittent protection to these mobile species can enhance abundance and possibly increase harvests (Polacheck 1990; Sumaila 1998; Maury and Gascuel 1999).

The major goal of this study is to investigate the potential of MPAs for the management of species with a moderately mobile adult life phase. The model used is that of the penaeid prawn *Penaeus latisulcatus* in Gulf St. Vincent, South Australia. Fishing of this stock only occurs for 35–40 nights a year from 10 boats, with effort concentrated in deeper regions of the gulf (30–40 m), although most areas below a depth of 20 m are lightly fished. Fishing effort data are available for 121 fishing blocks (30 nm^2) and show that in the 1997–1998 fishing season, only four blocks were fished sufficiently intensely that on average the entire area of the block was swept once (J.E. Tanner, unpublished data). Effort restrictions mean that the fleet is only capable of trawling considerably less than 10% of the gulf in any one year, thus creating a de facto reserve, albeit one that can change location from year to year. While this seemingly results in a very low level of exploitation, the stock has only just recovered from collapse and a subsequent 2-year closure, with fishing recommencing in December 1993. Due to the low exploitation level, it should be possible to establish marine reserves as a habitat conservation measure with little effect on the fishery.

Penaeus latisulcatus in South Australia is at the southern limit of its range and as a consequence has a much slower life cycle than that of most exploited penaeid populations, making it more vulnerable to overexploitation. Spawning occurs over the summer period and is concentrated in December–January (King 1977). There is some evidence to suggest that populations of this species further north exhibit multiple spawnings in a season (Penn 1980), but whether this holds true for the study population is unknown. After spawning, juveniles undergo a planktonic phase that lasts for approximately 4 weeks, after which they settle into inshore nursery areas, predominantly intertidal and shallow subtidal mud flats between mangroves and seagrass beds (Kangas 1999). Juveniles spend slightly over a year in these nursery grounds, with most migrating to deeper water in about March of the following year, at which time they begin to recruit to the fishery. First spawning generally occurs at 2 years of age, and few individuals live past an age of 5 years. This contrasts with penaeid populations in tropical areas that have life spans on the order of 12–18 months. Adults appear to be highly migratory within Gulf St. Vincent and its approaches (an area of ~13 000 km^2), with a tendency to inhabit the northern portion during the summer spawning season and to move south over winter. There is, however, little exchange with other nearby populations (Kangas 1999).

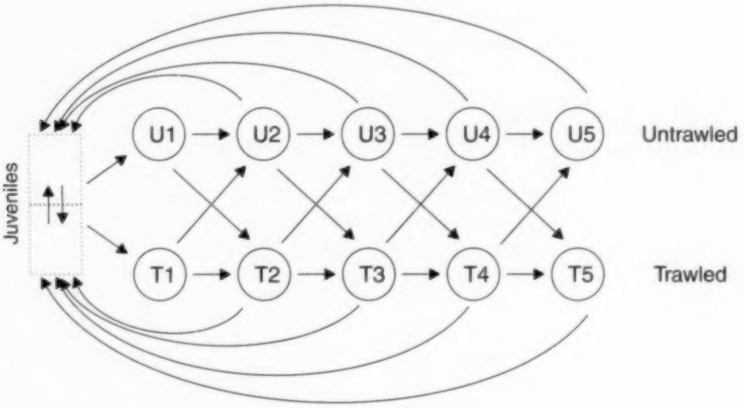
A simple multihabitat model of the population dynamics of *P. latisulcatus* is used to examine the consequences of varying the proportion of habitat trawled for long-term harvest rates. The model used differs from most previously published models in that both adults and juveniles disperse independently and at variable rates, juveniles occupy a different habitat than adults, and fishing mortality is spatially partitioned. This model also incorporates both a stock–recruitment relationship and density-dependent juvenile survival, both of which have important consequences for model output. The only difference between trawled and untrawled habitat in the model is the presence of fishing mortality in trawled areas. Importantly, it is assumed that both habitat types are equally suitable for adult prawns and that trawling has no detrimental (or beneficial) environmental impacts. Preliminary data from experimental work indicate that light trawling has no effect on subsequent catches in the trawled area, suggesting that *P. latisulcatus* does not perceive a degradation in habitat quality from such trawling (J.E. Tanner, unpublished data). Exchange of adults between the two habitats occurs once a year, and juveniles can recruit to either habitat type irrespective of their origin. The model is used to determine the optimal proportion of the habitat to trawl and to examine the consequences of changing fishing mortality. The assumptions of the model are tested via a sensitivity analysis to help determine what aspects of the life history are most crucial in determining population dynamics.

Multihabitat model

The model developed here is based on the age-structured Leslie matrix model (reviewed in Caswell 1989) with five 1-year age-classes. Two adult habitat types (or management zones), trawled and untrawled, are explicitly included in the model, while juveniles occupy a third nursery habitat in shallow untrawled waters (Fig. 1). To incorporate both adult habitats, the transition matrix is divided into four submatrices, with those at the top left and bottom right corners being Leslie matrices describing dynamics within untrawled and trawled habitats, respectively, while the top right and bottom left submatrices describe migration between habitats. Because juveniles occur in a third distinct habitat and occupy these nursery areas for approximately 1 year, it is assumed that they are completely mixed and recruit to adult habitats in proportion to habitat area, irrespective of whether they were derived from adults in the trawled or untrawled area (i.e., recruitment is uniform over the entire habitat). Reproduction is assumed to occur immediately postcensus (on 1 January each year), and thus, juveniles recruit straight into adult habitat as 1+ individuals just prior to the next census.

Adult movement between habitats in the model occurs at the end of the census interval. Because this is a highly mobile species and the population being studied occurs within a single embayment (of ~13 000 km^2), the model assumes complete mixing of the adult population every year resulting in a uniform distribution across the habitat (i.e., all adults move, but not all change habitats in the process). Changing the frequency of movement (e.g., to a monthly basis) had only minimal effects on model output (J.E. Tanner, unpublished data). Unless otherwise stated, natural mortality in both habitats is 1.25·year^{-1} (Morgan 1995), while prawns in

Fig. 1. Life cycle graph and transition probability matrix for *P. latisulcatus* existing in two habitats (trawled and untrawled). Adults progress through a series of age-classes (1–5) in each habitat and may also migrate between habitats at the end of each year. Individuals in age-class 2 and greater (in either habitat) may reproduce at the beginning of the year, resulting in juveniles that occur in distinct nursery areas and that recruit into either adult habitat at the end of the year. Fecundities (number of juvenile recruiting into age-class 1) for individuals in each age-class are given in the top row of each submatrix, while survival and migration probabilities are on the subdiagonals. $P_{X \to Y}$ = Probability that an individual that migrates from habitat X to Y survives for the census interval (1 year). $S_{X \to Y}$ = Probability that an individual in habitat X migrates to habitat Y. $S_U = e^{-M}(1 - P_{U \to T})$; $S_T = e^{-(M+F)}(1 - P_{T \to U})$; $S_{U \to T} = e^{-(M+F/2)}P_{U \to T}$; $S_{T \to U} = e^{-(M+F/2)}P_{T \to U}$.



$$
\begin{array}{c|ccccc|ccccc|}
 & & & \text{Untrawled} & & & & & \text{Trawled} & & \\
\hline
 & 0 & 4.98 & 9.03 & 11.11 & 12.68 & 0 & 4.98 & 9.03 & 11.11 & 12.68 \\
 & S_U & 0 & 0 & 0 & 0 & S_{T \to U} & 0 & 0 & 0 & 0 \\
\text{Untrawled} & 0 & S_U & 0 & 0 & 0 & 0 & S_{T \to U} & 0 & 0 & 0 \\
 & 0 & 0 & S_U & 0 & 0 & 0 & 0 & S_{T \to U} & 0 & 0 \\
 & 0 & 0 & 0 & S_U & 0 & 0 & 0 & 0 & S_{T \to U} & 0 \\
\hline
 & 0 & 4.98 & 9.03 & 11.11 & 12.68 & 0 & 4.98 & 9.03 & 11.11 & 12.68 \\
\text{Trawled} & S_{U \to T} & 0 & 0 & 0 & 0 & S_T & 0 & 0 & 0 & 0 \\
 & 0 & S_{U \to T} & 0 & 0 & 0 & 0 & S_T & 0 & 0 & 0 \\
 & 0 & 0 & S_{U \to T} & 0 & 0 & 0 & 0 & S_T & 0 & 0 \\
 & 0 & 0 & 0 & S_{U \to T} & 0 & 0 & 0 & 0 & S_T & 0 \\
\hline
\end{array}
$$

the trawled habitat are also exposed to fishing mortality. In this case, natural mortality is constant for all age-classes. Catchability is also constant over all age-classes that have recruited to the fishery, with recruitment to the fishery occurring at age 2+ unless otherwise stated. Further, it is assumed that fishing mortality is uniform over the trawled area and that all animals that die due to fishing are retained unless otherwise stated. The realised harvest is $F/(M + F)$ of the animals in each age-class that fail to survive. All mortality occurs after reproduction and before migration and can thus be considered an instantaneous process.

To calculate age-specific fecundities, the average carapace length of females in each age-class was determined from Morgan (1995). These average carapace lengths were then used to determine ovary weight according to the relationship (Penn 1980)

$$(1)\quad \text{Ovary weight} = 6.95 \times 10^{-5} \times \text{carapace length}^{2.916}$$

and then fecundities based on the value of 88 494 ova per gram of ovary (Penn 1980). The link between fecundity and recruitment was calculated using published stage duration times and mortalities. After spawning, several distinct phases are passed through before the juveniles settle into the nursery habitat. The egg phase at 29°C lasts for 12.5 h, followed by a series of naupliar stages that last for 36.5 h, a protozoea of 2.96 days, and a mysis of 3 days (Shokita 1984). However, Carrick (1996) studied the protozoeal and mysis stages at 18°C (in Spencer Gulf, close to Gulf St. Vincent) and found that they lasted for 8 and 12 days, respectively (three to four times as long as found by Shokita 1984). Thus, the durations for the two earlier stages (egg and nauplius) were multiplied by 3.5 to correct for lower water temperatures and thus slower development times (becoming 1.8 and 5.3 days, respectively). These development times are consistent with those for other species in the genus for which development at similarly low temperatures has been studied (Dall et al. 1990). Using data from Carrick (1996), protozoea have a mortality of $0.27 \cdot \text{day}^{-1}$ and mysis $0.07 \cdot \text{day}^{-1}$. Both eggs and the nauplius were arbitrarily assigned a mortality of $0.5 \cdot \text{day}^{-1}$. The remaining 48 weeks of the first year of life were spent as juveniles in the nursery grounds where mortality was density dependent. Overall, survival of eggs to juveniles 4 weeks later was 0.0014.

Density dependence in juvenile mortality was introduced to more accurately model population dynamics. As with many fished stocks, it was assumed that density dependence occurs primarily in the juvenile stage of the life cycle, as this is the stage with greatest abundance and least amount of available habitat. Kangas (1999) estimated weekly juvenile mortality to be related to density according to the equation

$$(2)\ \text{Mortality} = 0.032 + 0.1 \times \text{density}$$

where density was the number of juveniles in 1 m^2. This relationship was incorporated into the recruitment calculations assuming that 150 km^2 of juvenile habitat was available (Kangas 1999) and that settlement density was constant throughout this area. The population size of the juvenile phase was calculated on a weekly basis using the above mortality rate for 48 weeks following the 4-week egg/larval period in between each annual iteration of adult population size.

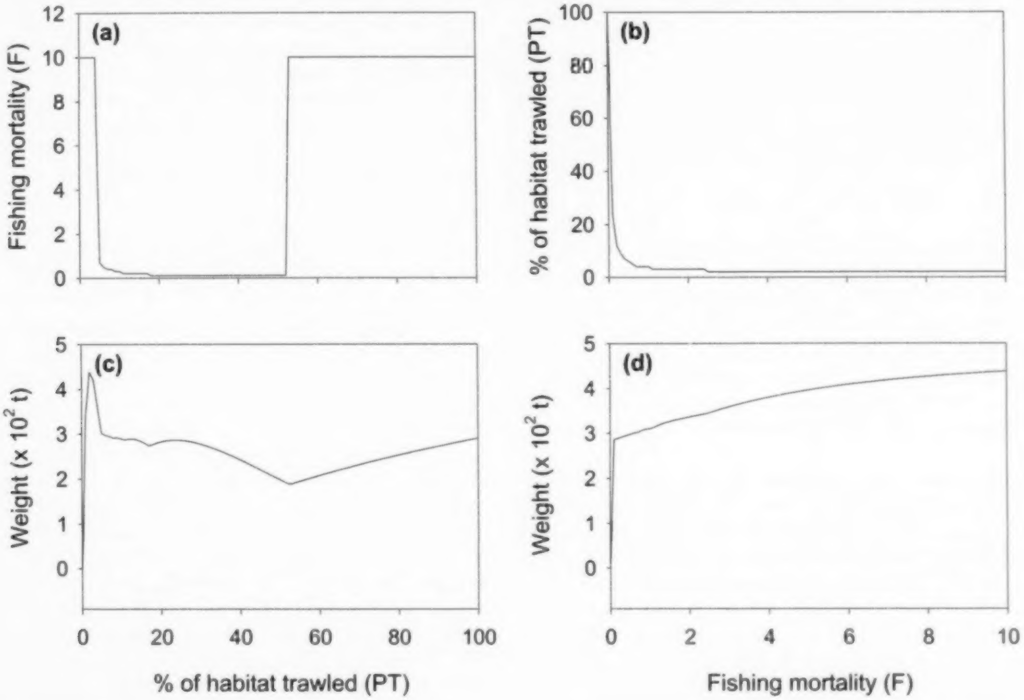
The above model was simulated for 200 years starting with 100 000 individuals of age 1+ in the untrawled area to determine how harvest weight (in the final year) responded to changes in the percentage of the habitat trawled (PT) (from 0 to 100% in 1% increments) and fishing mortality (from 0 to 10 in increments of 0.1). This starting population size produced equilibrium harvest rates consistent with current yearly harvests (~300 $t \cdot year^{-1}$), and subsequent analyses starting with several different estimates of the current stock structure produced the same results (J.E. Tanner, unpublished data). Two hundred years was more than sufficient for the model to equilibrate, which normally occurred in about 50 years. Results are presented in terms of yield by multiplying the number of harvested animals caught in each age-class by age-specific weights and assuming that equal numbers of males and females were caught. For females, weights used were 31, 66, 93, 110, and 120 g for age-classes 1–5, respectively, while for males, weights were 15, 32, 45, 53, and 58 g, respectively. After determining the optimal fishing mortality and percentage of habitat to trawl (with optimal defined as that percentage that resulted in maximum yield in year 200 of the model simulations), I investigated how yield responded to changes in natural adult mortality, adult and juvenile mixing, the strength of juvenile density dependence, and target harvest age by altering these factors independently. The importance of density dependence in the juvenile stage was also examined by setting recruitment to age 1+ constant at 1.4×10^9 $animals \cdot year^{-1}$, the number in this age-class at equilibrium in the model with no fishing. This scenario assumes that harvesting the adult population does not result in a decrease in recruitment (i.e., there is no stock–recruit relationship). Reduced mixing between habitat types was simulated by requiring that a given percentage (from 0 to 100) of animals remain in their current (or parent's) habitat while allowing the rest to mix randomly. Increased mixing was simulated in a similar fashion, except a given percentage of animals were forced to change habitats. Sensitivity analyses for changes in both adult and juvenile migration were conducted for two potential harvest strategies (F = 10, PT = 2% and F = 0.7, PT = 5%).

Results

Under the situation described by the model, the obtainable harvest was greatest when only 2% of the habitat was trawled, but with an extremely high fishing mortality in the fished area ($F \geq 10$, which was the cutoff in the model) (Fig. 2*c*). Catches under this harvest regime would, however, be very dependent on knowing precisely how much of the habitat had been trawled, as only slight changes in this variable result in substantially lower catches (as indicated by the close spacing of the lines of equal harvest in the horizontal direction in Fig. 3). Harvests of ~70% of the maximum could be obtained by fishing a larger proportion of the habitat at a lower fishing mortality (Fig. 2*c*), which has the advantage that harvest is not so dependent on precise estimates of the proportion of the habitat that has been trawled (isolines in Fig. 3 are widely spaced in the horizontal direction). For example, it only takes an increase in the trawled area to 5% of the habitat to bring the optimal fishing mortality down to ~0.7 (Fig. 2*a*). Conversely, at very low fishing mortality, the majority of the habitat must be fished to maximise harvest, but it only requires a slight increase in fishing mortality to greatly reduce the area fished (Fig. 2*b*). As would be expected, as the proportion of habitat trawled increases, the harvest depends more and more on a precise knowledge of fishing mortality, and the extent of the area trawled becomes unimportant (Fig. 3). Interestingly, once harvest peaks for any given fishing mortality or percentage of habitat trawled, it does not decrease monotonically. Instead, under very intensive harvesting regimes, it begins to increase again, although under the range of scenarios modelled, it never reaches any more than about two thirds the maximum obtainable (Figs. 2 and 3). The sudden increase in optimal fishing mortality when just over 50% of the habitat is trawled (Fig. 2*a*) occurs because the reproductive value of any unharvested adults becomes less than their current value if harvested immediately (remembering that all animals have a chance to reproduce before being subjected to fishing). It must be kept in mind when considering these results that fishing mortality only applies to the area actually swept by the nets (i.e., it equates to catchability). Under this definition, the actual fishing mortality for the fishery is probably in the range of 2–3 (5–14% of animals escape capture), although no data on this are available.

A sensitivity analysis of natural mortality indicated that slight changes could dramatically alter the response of harvest to changes in the percentage of habitat trawled and fishing mortality. The natural mortality rate used in the model (M = 1.25) is at the high end of the range of estimated mortality. Increasing this value further (to M = 1.3) resulted in a qualitative change in model behaviour, with harvest increasing monotonically with both fishing mortality and the proportion of the habitat harvested, although maximum harvest declined by almost 50% (Fig. 4*a*). This pattern suggests that as much of the habitat as possible should be trawled as heavily as possible to obtain the maximum harvest. Such heavy fishing is possible because harvest does not occur until after first reproduction, and high natural mortality means that despite first-year reproductives having relatively low fecundity, as a group, they provide a large proportion of new juveniles. Density-dependent mortality of juveniles further smooths out any differences in initial recruitment. Decreases

Fig. 2. (*a*) Fishing mortality for which maximum harvest is obtained when a given percentage of habitat is trawled, (*b*) percentage of habitat trawled to maximise catch at each fishing mortality, (*c*) weight of catch when optimising fishing mortality for a given percentage of habitat trawled, and (*d*) weight of catch when optimising percentage of habitat trawled for a given fishing mortality.



in natural mortality, however, did not result in qualitative changes to model output, although they did result in harvest increasing to unrealistically high levels, suggesting that natural mortality does not drop much below 1.25 unless compensatory changes occur in other parts of the life cycle (Fig. 4*b*). Providing the true natural mortality is no greater than 1.25, a precise knowledge of its value is not needed to determine the best harvest regime to use (although it is important in estimating the actual catch that will be obtained under that regime). Changing the survival rate of eggs and larvae (in the first 4 weeks) had identical consequences. Again, it only required a slight reduction in the number of larvae recruiting into the nursery habitat to result in harvest increasing monotonically with fishing mortality and area trawled, while increasing recruitment increased harvest but did not alter the response to changes in the harvesting regime (J.E. Tanner, unpublished data).

Small changes in movement patterns between habitats could sometimes result in dramatic changes in obtainable harvest, but at other times, large changes in movement had little consequence for harvest (Fig. 5). If there is any tendency at all for adults to move between habitat types more often than expected by chance (i.e., if adult movement is >0.5), then the harvest decreases substantially, although if there is almost complete swapping of habitats each year (movement close to 1), it does begin to increase again. While such complete swapping is biologically unrealistic (especially when only a small proportion of the habitat is trawled), it is impossible to determine the exact range of realistic scenarios, so all possibilities are presented for completeness. When adults tend to

Fig. 3. Influence of adjusting the proportion of habitat trawled and fishing mortality on relative harvest weight of *P. latisulcatus* when $M = 1.25\cdot\text{year}^{-1}$ and both juveniles and adults mix randomly between habitats. Isolines indicate parameter combinations with equal harvest ($\times 10^2$ t), with close spacing of isolines indicating that small changes in the parameter cause large changes in harvest.

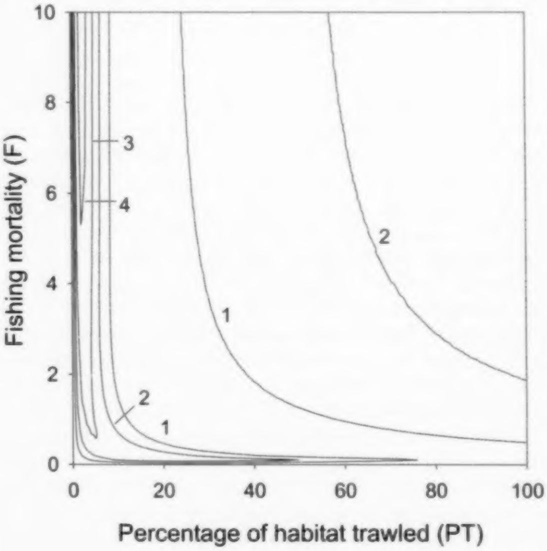


Fig. 4. Influence of natural mortality on modelled harvest ($\times 10^2$ t) of *P. latisulcatus*: (*a*) $M = 1.3$; (*b*) $M = 1.2$. Harvest for $M = 1.25$ is given in Fig. 3.

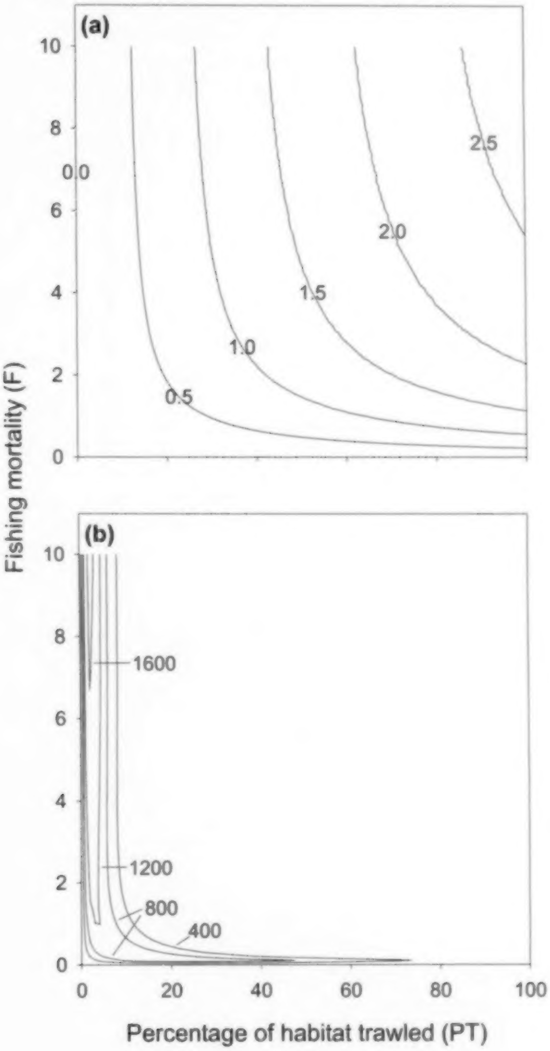
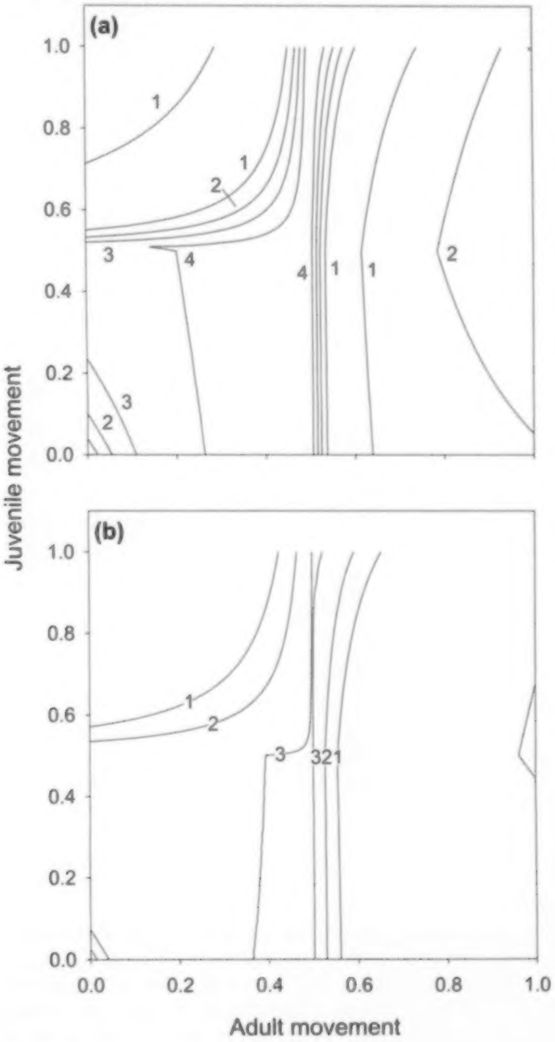


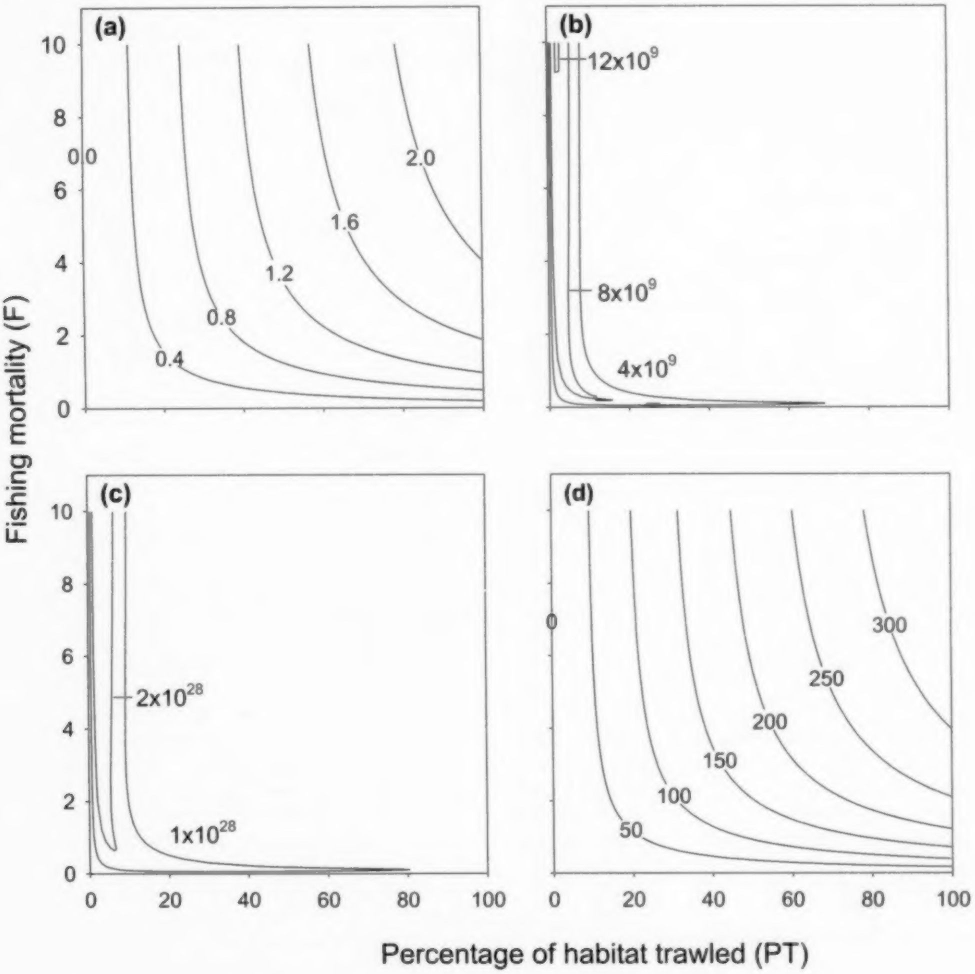
Fig. 5. Sensitivity of harvest weight ($\times 10^2$ t) of *P. latisulcatus* to changes in assumptions about adult and juvenile mixing under two different harvesting strategies. A migration rate of 0 indicates that there is no exchange between habitat types, 0.5 indicates random mixing based on the proportion of habitat occupied by each type, while 1 indicates that all individuals migrate to the opposite habitat. (*a*) $F = 10$, PT = 2%; (*b*) $F = 0.7$, PT = 5%.



remain in the same habitat from year to year, and juveniles recruit to the same habitat as their parents (movement <0.5 for both adults and juveniles), harvest remains at around the maximum unless there is very little exchange between the two habitats (i.e., unless there are actually two distinct populations). If, however, juveniles show a preference for recruiting to the opposite habitat to that of their parents, harvest again decreases substantially unless adults engage in perfectly random mixing. These patterns are consistent for a range of realistic harvest regimes ($F = 10$, PT = 2% and $F = 0.7$, PT = 5% are presented in Fig. 5, but similar patterns occurred for all PT < 10% when fishing mortality was at its optimum).

The response of harvest to changes in the assumption about juvenile density dependence was similar to the response to changes in natural mortality of adults. When density dependence was made stronger, harvest increased monotonically with both fishing mortality and the proportion of the habitat trawled (Fig. 6*a*). Reducing the strength of density dependence only resulted in a numerical response, however, with harvest increasing under a given fishing regime but still reaching a peak when only a small proportion of the habitat was trawled (Fig. 6*b*). If density dependence was removed from the model by setting juvenile mortality at $0.083 \cdot \text{week}^{-1}$, population size and harvest grew exponentially (Fig. 6*c*). Assuming constant recruitment to age 1+ (i.e., assuming no density dependence or stock–recruit relationship) had the same qualitative effect as increasing the

Fig. 6. Influence of adjusting the strength of density dependence in the juvenile phase on harvest weight ($\times 10^2$ t). (*a*) For 125% strength, the density value in eq. 2 is multiplied by 1.25, and (*b*) for 75% strength, it is multiplied by 0.75. (*c*) No density dependence; (*d*) constant recruitment to age 1+ (i.e., no stock–recruitment relationship). Figure 3 gives the situation when density dependence is not modified.



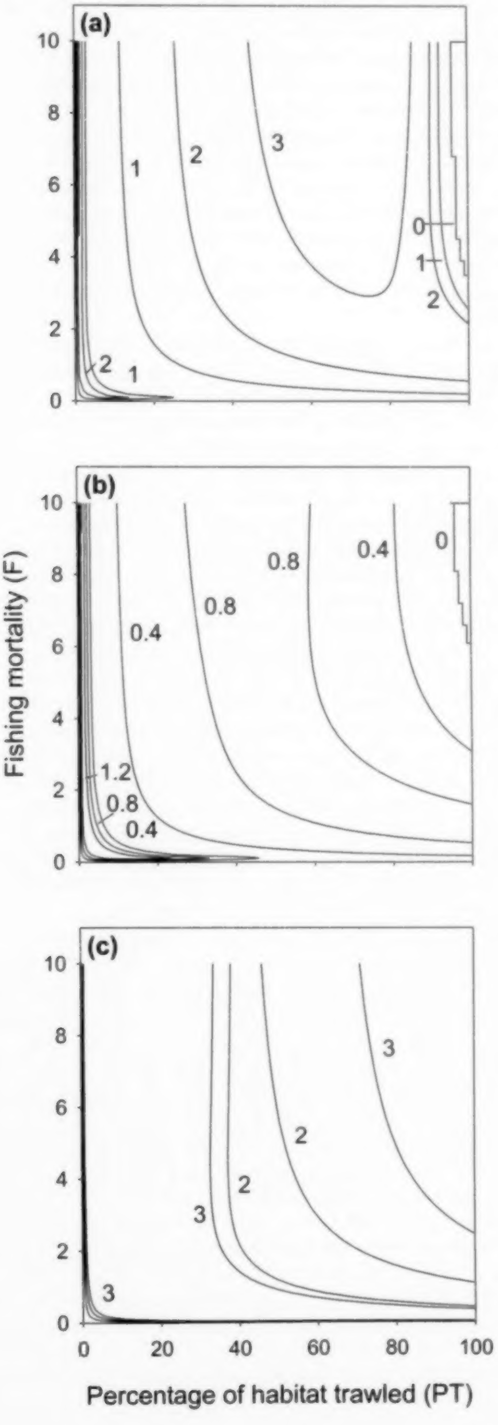
strength of density dependence, although harvest increased greatly (Fig. 6*d*). Again, most of these changes resulted in extremely unrealistic levels of harvest, indicating that the modified models contain incorrect assumptions and that the original equation used to represent density dependence is fairly realistic.

Changing the target age for recruitment to the fishery showed that the optimum harvest strategy was to target the 2+ age-class, as is currently the practise. Harvesting 1+ individuals resulted in harvest decreasing by about one third, although very intensive harvesting regimes could result in an increase in harvest back to almost the same level as when targeting 2+ individuals (Fig. 7*a*). However, if 2+ animals are targeted, but a substantial proportion of 1+ animals die in the process (either from damage sustained in escaping from the net or from being caught and discarded), the harvest suffers. A fishing mortality of $F/2$ for the 1+ age group (but no actual retention of these in the catch) results in a 60% decrease in the harvest (which is more than from actively fishing the 1+ group) (Fig. 7*b*). Switching to harvesting 3+ and older animals only also resulted in declines in harvest, although the response to changes in the harvest regime was qualitatively similar to changes when harvesting 2+ individuals (Fig. 7*b*).

Discussion

The model developed here indicates that restricting trawling to only part of the habitat of adult *P. latisulcatus* can potentially result in greater catches than harvesting the entire habitat. The current fishing strategy, based on effort restrictions, is to fish a small proportion of the gulf fairly intensively. Although the catchability of prawns in this fishery is not known, it is likely to be high, and thus, fishing mortality of the targeted age-classes in the fished area (i.e., the area swept by the nets) is probably very high. Catch and effort re-

Fig. 7. Effect of modifying age of entry into the fishery on relative harvest (×10^2 t) of *P. latisulcatus* when $M = 1.25\cdot\text{year}^{-1}$. (*a*) 1+ individuals are fished; (*b*) 2+ individuals are actively fished but 1+ animals experience a fishing mortality of $F/2$, although they are not retained; (*c*) entry into the fishery is delayed to age 3+. Figure 3 gives the situation for entry at age 2+.



cords indicate that from 1993 to 1998, between 200 and 450 km^2 a year was trawled (assuming a trawl speed of 3 kn, with nets sweeping a swathe 20 m wide). This equates to between 1.5 and 3.5% of the gulf if no areas are trawled more than once. Thus, the fishing strategy that has been adopted appears to be as close to optimal as it is possible to get with our current level of knowledge. Such low levels of fishing also mean that it should be possible to introduce marine reserves into Gulf St. Vincent as a conservation tool with little or no conflict with the fishing industry, provided fishers are consulted at an early stage.

Importantly, the incorporation of a stock–recruitment relationship into the model (through adult fecundity) produced qualitatively different behaviour than the scenario modelled with constant recruitment to age 1+ (i.e., to what is essentially a yield per recruit model). While yield per recruit models are likely to do a good job in situations where recruitment is controlled by factors extrinsic to the stock being modelled (e.g., environmental conditions, limited nursery areas, or large sources of recruits from outside the stock under consideration), if recruitment is determined largely by the total fecundity of the stock, then yield per recruit models may result in incorrect conclusions being drawn. Of course, the real situation for the current stock (and many others) is likely to lie somewhere in between, with a stock–recruitment relationship obscured to some extent by environmental noise, and it is important to determine how this noise might influence population dynamics and harvest.

While maximum harvest under the assumptions of the model occurs when only ~2% of the habitat is fished, maintaining this harvest requires very precise knowledge of what proportion has been trawled, as indicated by the close spacing of the lines indicating constant harvest when percentage of habitat trawled changes at high fishing mortality. As we do not have detailed knowledge on the habitat requirements of adult prawns (although we do know that they can be found over most of the gulf), determining this proportion precisely is not possible, making such a harvest regime very risky. It only requires a shift in strategy to trawling a slightly larger area (5–10%) with a lower fishing mortality (0.7–0.3) to make errors of a few percent in the proportion of habitat trawled considerably less important (i.e., at $F = 10$, there is a large change in harvest moving from trawling 1% of the habitat to 3–5%, whereas at $F = 0.7$, the change moving from 3 to 5–7% is less). Catches will only be 70% of maximum under such a regime, but because the model is less sensitive to errors in the estimates of fishing mortality and percentage of habitat trawled, there is less risk of overharvesting. While it is also difficult if not impossible to estimate fishing mortality accurately, harvest under this later regime is also relatively insensitive to errors in fishing mortality. Manipulating fishing mortality is likely to be difficult, however, making it important to determine what areas are actually suitable habitats for this species. The high degree of sensitivity of the model to important parameters such as fishing mortality and percentage of habitat trawled, and the inherent unknowability of their values, means that it is vital to carefully monitor the stock and adopt a precautionary approach to management.

As is the case with most fishery models, this model is very sensitive to the value of natural mortality. To be conservative (i.e., maintain stock size at the risk of reduced harvest), I have used the maximum mortality that has been estimated for this stock ($M = 1.25$), although other estimates range as low as $M = 1$ (Morgan 1995). Within this range, the general qualitative predictions of the model hold, although quantitative predictions vary greatly and are therefore not reliable. In fact, when natural mortality drops to 1.2, harvest levels become unrealistically high due to a breakdown in density-dependent population regulation, and hence unrestricted population growth in the model. Similarly, the model was very sensitive to changes in recruitment to the nursery habitat and in the strength of juvenile density dependence, with both parameters being close to thresholds that qualitatively changed the behaviour of the model (for example, this study shows that stronger density dependence leads to harvest increasing monotonically with fishing pressure rather than reaching a peak at some intermediate level). Again, both of these parameters were chosen to be conservative (i.e., to avoid overharvesting), so it is unlikely (but not impossible) that these thresholds are in fact exceeded in the real population. In particular, the equation for juvenile density dependence assumes that all individuals in the trawl path were caught (Kangas 1999), and if this is not the case, actual density dependence will not be as strong, moving the population away from this threshold. Provided none of these thresholds were exceeded, the optimal harvest regime did not vary greatly (although catch did). This is in stark contrast with some other studies, where the optimal proportion of habitat to protect depended on the exact values of model parameters (e.g., Sladek Nowlis and Roberts 1998). That density dependence only occurs in the juvenile phase is a standard assumption of fishery models (e.g., Hastings and Botsford 1999), although model sensitivity to this assumption is rarely tested.

Like several other simulation studies (e.g., Polacheck 1990; Sumaila 1998; Guénette and Pitcher 1999), the model developed here shows that removing a large proportion of the habitat from access by the fishery is a potentially viable way of managing the fishery. In this particular instance, such an action would be somewhat redundant, as current effort restrictions have the effect of creating a defacto restriction on the amount of habitat trawled anyway. The model also supports the contention that an MPA by itself would be unlikely to serve as a sufficient restriction on fishing (Holland and Brazee 1996; Allison et al. 1998). Unless the MPA covered 97–98% of the habitat, unrestricted effort in the open area could still easily result in substantial decreases in realised harvest (in this study, harvest decreases at high fishing mortality when >3% of the habitat is trawled). Such a large area is needed because of the assumptions of complete mixing of both adults and juveniles, which means that declines in stock size in the harvested area rapidly translate into declines in the unharvested area.

A particularly important point to note is that while restricting harvest to 2+ individuals is predicted to maximise harvest, if substantial numbers of 1+ individuals are killed in the process but not retained, then this restriction is counterproductive. It thus becomes important to determine if 1+ animals that either pass through the net or are caught but discarded actually survive the event. As the gear used is relatively light, and the wings of the net are of a sufficiently large mesh size as to allow these smaller prawns to pass through, it is possible that these animals do have a high survival rate, with few being retained in the cod-end. Those actually caught, however, are likely to experience high mortality rates even if returned to the water, as they will have been tumbled around in the cod-end for up to 2 h and then subjected to considerable further damage when brought on deck. What is crucial to the maintenance of stock size (and thus yield) is the fact that high natural mortality rates in combination with harvesting only 2+ and older prawns (which have had a chance to reproduce) means that the population is dominated by young unfished individuals. As a result, even very intensive fishing of older animals has little influence on the population's reproductive output and subsequent recruitment to the fishery. In fact, the model indicates less than a 10% variation in the number of 1+ individuals present under the range of fishing regimes investigated (not shown).

The spatial arrangement of habitat patches is not considered in this study. Instead, it is assumed that prawns are able to choose freely between habitat types when they migrate. If suitable patches of habitat are relatively ephemeral, or the areas trawled change on a regular basis, the spatial arrangement of patches may not matter. In a generalised simulation study, Fahrig (1992) showed that the temporal scale of habitat patchiness was much more influential in determining population size than was the spatial scale. It was only when habitat patches persisted for long periods of time that spatial scale became important. Even if habitat patches are permanent, the spatial arrangement of habitat may not be important if there is high connectivity between patches (Fahrig 1998). This is likely to be true for *P. latisulcatus*, which has a highly mobile adult phase in relation to the distribution of the population as well as a dispersive juvenile phase. This suggests that under the assumption of a uniform habitat, it does not matter which areas are trawled and which are protected. It is probably more important, however, that the trawl areas do not change to a great extent on a yearly basis. Such yearly changes are possible under the current management regime, although fishers show a preference for fishing over previously trawled (and therefore clean) ground.

Most previous models looking at MPAs have assumed either that larvae disperse while adults are relatively sedentary (e.g., Botsford et al. 1993; Sladek Nowlis and Roberts 1998; Hastings and Botsford 1999) or that adults move and larvae recruit evenly across reserve and nonreserve areas (e.g., Polacheck 1990; Guénette and Pitcher 1999). Rarely are the consequences of varying both adult and juvenile dispersal abilities examined in a single model. The model used here suggests that for *P. latisulcatus*, both adult and juvenile migration interact to determine stock size and harvest. Juvenile migration only becomes important when adults are relatively sedentary (i.e., tend not to swap habitats as often as would be expected under the random mixing assumption). In this case, if they recruit to the habitat not occupied by their parents (and then stay there), the usefulness of reserves breaks down and the stock rapidly collapses. There is a large area of parameter space where exchange of both adults and juveniles between the two habitats is limited (i.e., when the stock starts to behave like a metapopulation) that maintains high harvest rates. This is in accord with a study by Man et al. (1995) that showed that reserves are particularly useful when a fished stock exists as a metapopulation.

It is particularly important to remember that this model does not include any environmental stochasticity. The addition of such stochasticity into population models almost always results in decreases in long-term modelled population size (but see Damman and Cain 1998; Tanner 2000), with consequent decreases in sustainable harvests. Lauck et al. (1998) demonstrated that as variation in the level of harvest increases, the probability of stock collapse can increase dramatically. In their model, even moderate levels of uncertainty caused collapse unless ~50% of the habitat was protected. Adding variation into demographic rates as well is likely to exacerbate the situation further and will be examined in detail in a later paper.

MPAs not only protect a stock from overexploitation, but may also increase the yield of certain fisheries (e.g., Russ and Alcala 1996). This study demonstrates that maximising long-term harvest requires protection of most of the habitat from exploitation, either directly by creation of a reserve or indirectly through effort controls. In the present situation, this occurs because there is a high level of exchange between harvested and protected areas and because recruits derived from protected areas can colonise harvested areas. Maury and Gascuel (1999) showed that such increases in yield occur chiefly in highly mobile species, as fish tend to be caught at older ages and thus larger sizes. Providing refuges for site-attached species can, however, decrease yield per recruit (which does not necessarily mean decreasing harvest if the reserve leads to increased recruitment, e.g., if a source area is protected), as individuals in the protected area never become available to the fishery. The effect is still present in species that migrate between protected and unprotected areas on an annual basis. This is particularly true for moderately to heavily fished stocks, whereas if the stock is lightly fished, an MPA is likely to have little positive affect and can substantially reduce yields (Holland and Brazee 1996). This is in line with the results presented here, where increases in fishing mortality resulted in maximum yield occurring when greater proportions of the habitat were protected. Conversely, at low levels of fishing effort, harvest decreased if large areas of the habitat were protected, albeit only slowly until well over 50% of the habitat was protected. Similarly, a number of field studies have shown that reserves can result in higher abundances of target species in nearby unprotected areas through spillover and that this increase in abundance may even compensate for the decreased area available for harvest (e.g., Alcala and Russ 1990; Yamasaki and Kuwahara 1990). This is particularly true when juvenile habitats are protected, which is a much more common scenario than the protection of adult habitat.

Acknowledgments

I would like to thank B. McDonald, M. Mangel, S. Shepherd, and several anonymous reviewers for their comments on an earlier version of this manuscript. Funding was provided by FRDC grant No. 1998/208.

References

Alcala, A.C., and Russ, G.R. 1990. A direct test of the effects of protective management on abundance and yield of tropical marine resources. J. Cons. Int. Explor. Mer, **46**: 40–47.

Allison, G.W., Lubchenco, J., and Carr, M.H. 1998. Marine reserves are necessary but not sufficient for marine conservation. Ecol. Appl. **8**: S79–S92.

Beverton, R.J.H., and Holt, S.J. 1957. On the dynamics of exploited fish populations. Chapman and Hall, London, U.K.

Bohnsack, J.A. 1998. Application of marine reserves to reef fisheries management. Aust. J. Ecol. **23**: 298–304.

Botsford, L.W., Quinn, J.F., Wing, S.R., and Brittnacher, J.G. 1993. Rotating spatial harvest of a benthic invertebrate, the red sea urchin, *Strongylocentrotus franciscanus*. *In* Proceedings of the International Symposium on Management Strategies for Exploited Fish Populations. *Edited by* G. Kruse, D.M. Eggers, R.J. Marasco, C. Pautzke, and T.J. Quinn. Alaska Sea Grant College Program Rep. No. 93-02. University of Alaska, Fairbanks, Alaska. pp. 409–428.

Brailovskaya, T. 1998. Obstacles to protecting marine biodiversity through marine wilderness preservation: examples from the New England region. Conserv. Biol. **12**: 1236–1240.

Buxton, C.D. 1992. The application of yield-per-recruit models to two South African sparid reef species, with special consideration to sex change. Fish. Res. **15**: 1–16.

Carr, M.H., and Reed, D.C. 1992. Conceptual issues relevant to marine harvest refuges: examples from temperate reef fishes. Can. J. Fish. Aquat. Sci. **50**: 2019–2028.

Carrick, N. 1996. Key factors which affect prawn recruitment and implications to harvesting prawn stocks. FRDC 91/3, Canberra, Australia.

Caswell, H. 1989. Matrix population models. Sinauer, Sunderland, Mass.

Dall, W., Hill, B.J., Rothlisberg, P.C., and Sharples, D.J. 1990. The biology of the Penaeidae. Adv. Mar. Biol. **27**: 1–461.

Damman, H., and Cain, M.L. 1998. Population growth and viability analyses of the clonal woodland herb, *Asarum canadense*. J. Ecol. **86**: 13–26.

Die, D.J., and Watson, R.A. 1992. A per-recruit simulation model for evaluating spatial closures in an Australian penaeid fishery. Aquat. Living Resour. **5**: 145–153.

Dugan, J.E., and Davis, G.E. 1993. Application of marine refugia to coastal fisheries management. Can. J. Fish. Aquat. Sci. **50**: 2029–2042.

Fahrig, L. 1992. Relative importance of spatial and temporal scales in a patchy environment. Theor. Popul. Biol. **41**: 300–314.

Fahrig, L. 1998. When does fragmentation of breeding habitat affect population survival? Ecol. Model. **105**: 273–292.

Guénette, S., and Pitcher, T.J. 1999. An age-structured model showing the benefits of marine reserves in controlling overexploitation. Fish. Res. **39**: 295–303.

Hastings, A., and Botsford, L.W. 1999. Equivalence in yield from marine reserves and traditional fisheries management. Science (Washington, D.C.), **284**: 1537–1538.

Holland, D.S., and Brazee, R.J. 1996. Marine reserves for fisheries management. Mar. Res. Econ. **11**: 157–171.

Horwood, J.W., Nichols, J.H., and Milligan, S. 1998. Evaluation of closed areas for fish stock conservation. J. Appl. Ecol. **35**: 893–903.

Kangas, M.V. 1999. Postlarval and juvenile western king prawn *Penaeus latisulcatus* Kishinouye studies in Gulf St. Vincent, South Australia with reference to the commercial fishery. Ph.D. thesis, University of Adelaide, Adelaide, Australia.

King, M.G. 1977. The biology of the western king prawn *Penaeus latisulcatus* Kishinouye and aspects of the fishery in South Australia. M.Sc. thesis, University of Adelaide, Adelaide, Australia..

Lauck, T., Clark, C.W., Mangel, M., and Munro, G.R. 1998. Implementing the precautionary principle in fisheries management through marine reserves. Ecol. Appl. **8**: S72–S78.

Man, A., Law, R., and Polunin, N.V.C. 1995. Role of marine reserves in recruitment to reef fisheries: a metapopulation model. Biol. Conserv. **71**: 197–204.

Mangel, M. 1998. No-take areas for sustainability of harvested species and a conservation invariant for marine reserves. Ecol. Lett. **1**: 87–90.

Maury, O., and Gascuel, D. 1999. SHADYS ('simulateur halieutique de dynamiques spatiales'), a GIS based numerical model of fisheries. Example application: the study of a marine protected area. Aquat. Living Resour. **12**: 77–88.

Morgan, G.R. 1995. Assessment, management and research support for the Gulf St. Vincent prawn fishery. South Australian Fisheries Management Series No. 12. Primary Industries South Australia, Adelaide, Australia.

Penn, J.W. 1980. Spawning and fecundity of the western king prawn, *Penaeus latisulcatus* Kishinouye, in Western Australian waters. Aust. J. Mar. Freshw. Res. **31**: 21–35.

Pennington, J.T. 1985. The ecology of fertilization of echinoid eggs: the consequences of sperm dilution, adult aggregation, and synchronous spawning. Biol. Bull. **169**: 417–430.

Polacheck, T. 1990. Year around closed areas as a management tool. Nat. Resour. Model. **4**: 327–354.

Russ, G.R., and Alcala, A.C. 1996. Marine reserves: rates and patterns of recovery and decline of large predatory fish. Ecol. Appl. **6**: 947–961.

Shokita, S. 1984. Larval development of *Penaeus* (*Melicertus*) *latisulcatus* Kishinouye (Decapoda, Natantia, Penaeidae) reared in the laboratory. Galaxea, **3**: 37–55.

Sladek Nowlis, J., and Roberts, C.M. 1998. Fisheries benefits and optimal design of marine reserves. Fish. Bull. (Washington, D.C.), **97**: 604–616.

Sumaila, U.R. 1998. Protected marine reserves as fisheries management tools: a bioeconomic analysis. Fish. Res. **37**: 287–296.

Tanner, J.E. 2000. Stochastic density-dependence regulates population size in a benthic marine invertebrate. Oecologia, **122**: 514–520.

Yamasaki, A., and Kuwahara, A. 1990. Preserved area to effect recovery of overfished Zuwai crab stocks off Kyoto Prefecture. Proceedings of the International Symposium on King and Tanner Crabs. Alaska Sea Grant College Program, University of Alaska, Fairbanks, Alaska. pp. 575–578.

A new model of growth back-calculation incorporating age effect based on otoliths

Kentaro Morita and Takashi Matsuishi

Abstract: We developed a simple back-calculation model that includes the age effect in which otolith size increases continuously during no-growth periods. To evaluate the validity of our proposed model, we back-calculated the past fish lengths and growth rates using the new model and seven traditional back-calculation models (scale-proportional hypothesis (SPH), body-proportional hypothesis (BPH), Fraser–Lee, biological intercept, nonlinear SPH, nonlinear BPH, and modified Fry) using the otoliths of individually tag-recaptured white-spotted char (*Salvelinus leucomaenis*). The estimated fish lengths corresponded well to observed fish lengths for simple traditional (SPH, BPH, and Fraser–Lee) and the new models. However, the back-calculated growth rates did not correspond to observed growth rates except for the new model. All previous models had considerable bias; growth rates of slow-growing fish were overestimated. As our model incorporating the age effect did not show such bias, this bias would be attributed to the age effect. Our proposed back-calculation model incorporating the age effect should be useful to estimate past growth rates at the individual level.

Résumé : On trouvera ici un modèle simple de rétro-calcul qui inclut l'effet de l'âge dans lequel la taille de l'otolithe continue d'augmenter durant les périodes d'absence de croissance corporelle. Pour éprouver la validité du modèle que nous proposons, nous avons déterminé par rétro-calcul les longueurs et les taux de croissance passés d'après les otolithes chez des Ombles à taches blanches (*Salvelinus leucomaenis*) marqués individuellement et recapturés. Nous avons utilisé notre nouveau modèle et sept modèles traditionnels de rétro-calcul (l'hypothèse de la proportionnalité à l'écaille (SPH), l'hypothèse de la proportionnalité au corps (BPH), la méthode de Fraser–Lee, la méthode de l'intercept biologique, la SPH non-linéaire, la BPH non-linéaire et la méthode de Fry modifiée). Les longueurs estimées des poissons correspondaient bien aux longueurs observées lorsque nous utilisions les modèles traditionnels simples (SPH, BPH et Fraser–Lee) et le nouveau modèle. Cependant, les taux de croissance obtenus par rétro-calcul ne correspondaient pas aux taux observés, sauf avec le nouveau modèle. Tous les modèles traditionnels généraient des erreurs importantes et les taux de croissance des poissons à croissance lente étaient surestimés. Puisque notre modèle de rétro-calcul, qui inclut l'effet de l'âge, ne génère pas de telles erreurs, nous attribuons l'erreur à l'effet de l'âge. Notre modèle devrait s'avérer utile pour estimer les taux antérieurs de croissance individuelle.

[Traduit par la Rédaction]

Introduction

Back-calculation of fish lengths from otoliths or scale annuli is a useful tool for fisheries scientists. Until recently, various studies have used back-calculation models to estimate the past fish length and growth (reviewed by Francis 1990). Although many back-calculation models have been proposed and their accuracy compared (Smedstad and Holm 1996; Horppila and Nyberg 1999; Klumb et al. 2001), most previous models are based on the assumption that fish growth is proportional to otolith (or scale) growth. However, many studies have recognized that fish growth and otolith growth could be uncoupled to some extent. Two primary causes are suggested: (*i*) the "growth effect" in which otoliths from slow-growing fish are larger than those of fast-growing fish of the same size (e.g., Reznick et al. 1989; Campana 1990) and (*ii*) the "age effect" in which the otolith increases in size continuously, even though somatic growth is zero (e.g., Mugiya 1990; Secor and Dean 1992; Holmgren 1996). Note that the increase in otolith size in nongrowing fish (age effect) is consistent with slow-growing fish having a larger otolith growth relative to somatic growth (growth effect). Thus, both growth effect and age effect have similar properties and are not mutually exclusive.

We hypothesize that using the traditional back-calculation models would overestimate fish growth rates for slow-growing fish because the otolith increases in size substantially, even though somatic growth is little (age effect or growth effect). In this study, we propose a simple back-calculation model incorporating the age effect to address the bias resulting from the uncoupling of fish growth and otolith growth as mentioned above. To evaluate the validity of our back-calculation model, we compared the outcome of our model with fish length and growth rate observed in the field using the otoliths of individually tag-recaptured white-spotted char (*Salvelinus leucomaenis*). To evaluate whether our model is better than previous ones, we also compared different models.

Received February 8, 2001. Accepted July 4, 2001. Published on the NRC Research Press Web site at http://cjfas.nrc.ca on August 25, 2001.
J16208

K. Morita[1] and T. Matsuishi. Faculty of Fisheries, Hokkaido University, Hakodate 041-8611, Japan.

[1]Corresponding author (e-mail: morita@fish.hokudai.ac.jp).

DOI: 10.1139/cjfas-58-9-1805

Methods

Back-calculation models

Traditional models

Most previous back-calculation models assumed that fish growth and otolith (or scale) growth are proportional (reviewed by Francis 1990). Several linear and nonlinear back-calculation models have been proposed. The most common back-calculation models are based on the following formulae:

$$O = a + bL \tag{1}$$

$$L = c + dO \tag{2}$$

$$O = uL^{v} \text{ or } \ln O = \ln u + v\ln L \tag{3}$$

$$L = wO^{k} \text{ or } \ln L = \ln w + k\ln O \tag{4}$$

where O is otolith length, L is fish body length, and a, b, c, d, u, v, w, and k are constants obtained from regression analyses. The most common back-calculation models are as follows. Scale-proportional hypothesis (SPH):

$$L_t = -\frac{a}{b} + \left(L_T + \frac{a}{b}\right)\frac{O_t}{O_T} \tag{5}$$

Body-proportional hypothesis (BPH):

$$L_t = \frac{c + dO_t}{c + dO_T} L_T \tag{6}$$

Fraser–Lee model:

$$L_t = c + (L_T - c)\left(\frac{O_t}{O_T}\right) \tag{7}$$

Nonlinear scale-proportional hypothesis (nonlinear SPH):

$$L_t = \left(\frac{O_t}{O_T}\right)^{\frac{1}{v}} L_T \tag{8}$$

Nonlinear body-proportional hypothesis (nonlinear BPH):

$$L_t = \left(\frac{O_t}{O_T}\right)^{k} L_T \tag{9}$$

In these models, L_t is the back-calculated fish body length at age t, L_T is the fish body length at the time of capture T, O_t is otolith length at annulus t, O_T is otolith length at the time of capture T, a, and b are constants as in eq. 1, c and d are constants as in eq. 2, v is a constant as in eq. 3, and k is a constant as in eq. 4. Campana (1990) modified the Fraser–Lee model using a biological intercept as follows:

$$L_t = L_T + \frac{O_t - O_T}{O_T - O_0}(L_T - L_0) \tag{10}$$

where O_0 and L_0 are the observed initial otolith and fish body length (i.e., biological intercept). The biological intercept method has been widely used in recent studies (Suthers and Sundby 1996; Fukuwaka and Kaeriyama 1997; Zenitani 1999) because it has a number of useful features. A useful feature of this method is that it does not require regressions and it can address growth effect when the intercept of regression is estimated (Campana 1990).

Recent models

Sirois et al. (1998) developed a back-calculation model incorporating growth effect, specifically the variation of growth rate over time. The proposed back-calculation model (time-varying growth (TVG)) is

$$L_t = L_0 + \sum_{i=1}^{t}[W_i + R(W_i - W)]\frac{L_T - L_0}{O_T - O_0} \tag{11}$$

where W_i is the otolith increment width at age i, W is the mean otolith increment width, and R is the parameter of growth effect; R is the slope from the regression of the slope of the fish length and otolith length relationship on absolute growth rate in fish length (details in Sirois et al. 1998).

Vigliola et al. (2000) developed a back-calculation model incorporating a biological intercept with the Fry model. The proposed back-calculation model is based on the following nonlinear formula:

$$L = L_0 - \mu O_0^{\omega} + \mu O^{\omega} \tag{12}$$

where μ and ω are constants obtained from nonlinear regression analysis. The proposed back-calculation model (modified Fry) is

$$L_t = \varphi + \exp\left[\ln(L_0 - \varphi) + [\ln(L_T - \varphi) - \ln(L_0 - \varphi)] \times \frac{\ln O_t - \ln O_0}{\ln O_T - \ln O_0}\right] \tag{13}$$

where φ is the fish body length at otolith formation, $\varphi = L_0 - \mu O_0^{\omega}$. As eq. 12 could be fitted using O or L as the independent variables, two values of φ could be derived. The average value of these estimates was used in the above back-calculation model (details in Vigliola et al. 2000).

A new model incorporating age effect

As otolith growth is a conservative process with otoliths increasing continuously during starvation or negative somatic growth (Secor and Dean 1992; Holmgren 1996; Barber and Jenkins 2001), we assume that the otolith increases with both fish body length and age as follows:

$$O = \alpha + \beta L + \gamma t \tag{14}$$

where O is otolith length, L is fish body length, t is fish age, and α, β, and γ are constants obtained from multiple regression analysis. If we assume that the deviation of the otolith length of a fish from the average for that fish length and age is relatively the same throughout the life of a fish (i.e., SPH), we have the following back-calculation model (see Appendix):

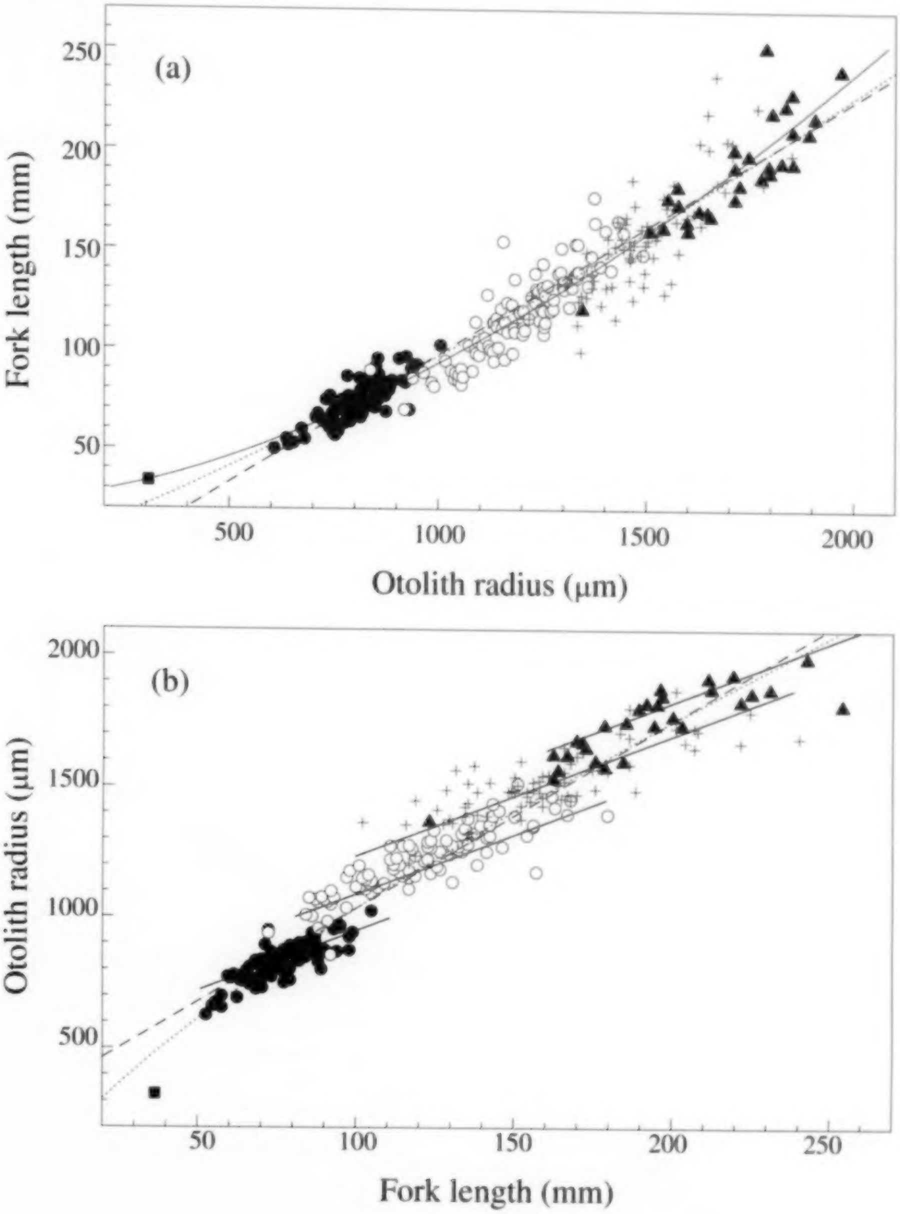
$$L_t = -\frac{\alpha}{\beta} + \left(L_T + \frac{\alpha}{\beta} + \frac{\gamma}{\beta}T\right)\frac{O_t}{O_T} - \frac{\gamma}{\beta}t \tag{15}$$

where L_t is the back-calculated fish body length at age t, L_T is the fish body length at the time of capture T, O_t is the otolith length at annulus (age) t, O_T is the otolith length at the time of capture T, and α, β, and γ are constants as in eq. 14.

Application of the models

We applied growth back-calculation models to the otolith of white-spotted char. The char otoliths selected for this study originated from previous field experiments in the Haraki River System, Hokkaido Island, Japan (Morita et al. 2000; Morita and Yamamoto 2001). In July 1997, a total of 200 fry (age 0+, mean fork length ±

Fig. 1. Relationships between (*a*) fork length and otolith radius and (*b*) otolith radius and fork length of white-spotted char. Solid circles, age 0+; open circles, age 1+; plus signs, age 2+; triangles, age 3+; square, biological intercept. Figure 1*a* regression lines: solid line, modified Fry model; dashed line, Fraser–Lee model or BPH; dotted line, nonlinear BPH. Figure 1*b* regression lines: solid lines, Morita–Matsuishi model proposed here; dashed line, SPH; dotted line, nonlinear SPH.



SD = 53.2 ± 5.9 mm) were collected and transplanted at the experimental section in a natural river where fishes were absent. In July 1998, a total of 83 fish of age 1+ were recaptured, fork lengths measured (118 ± 18 mm), and the fish tagged individually using numbered plastic anchor tags and then released immediately. In October 1999, a total of 74 fish of age 2+ were recaptured that included 43 tagged fish. In October 2000, a total of 29 fish of age 3+ were recaptured that included 16 tagged fish. The recaptured fish were measured for fork length (age 2+, 159 ± 28 mm; age 3+, 193 ± 28 mm) and the otoliths were removed. We also collected 82 fish of age 0+ in October 1997 and 94 fish of age 1+ in October 1999 when conducting different field experiments. These fish were measured for fork length (age 0+, 75 ± 12 mm; age 1+, 125 ± 20 mm) and the otoliths were removed. We measured the otolith radius at capture and the otolith radius for each annulus for both right and left otoliths using image analysis. The average of the two measures was used as the otolith radius of an individual. Biological intercept data were provided by S. Yamamoto (National Research

Fig. 2. Relationships between back-calculated fork length and observed fork length using SPH, BPH, Fraser–Lee, biological intercept, nonlinear SPH, nonlinear BPH, modified Fry, and Morita–Matsuishi models. Dashed lines, 1:1 relationships; solid lines, fitted regressions. Regression equations and r^2 values are given in each panel. Asterisks denote significant differences of the slopes and intercepts from 1 and 0, respectively, based on t tests ($^*p < 0.01$; $^{**}p < 0.001$).

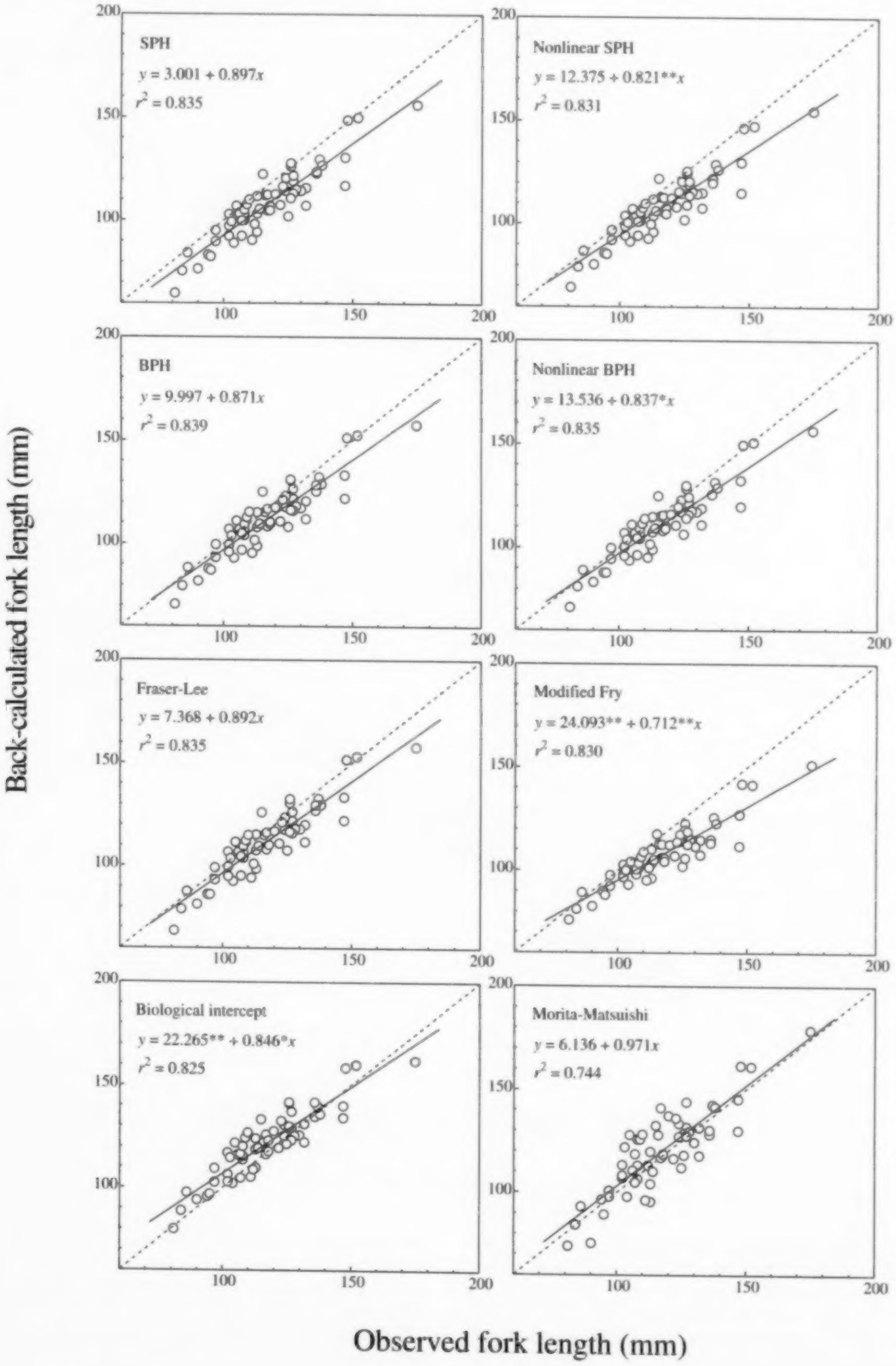
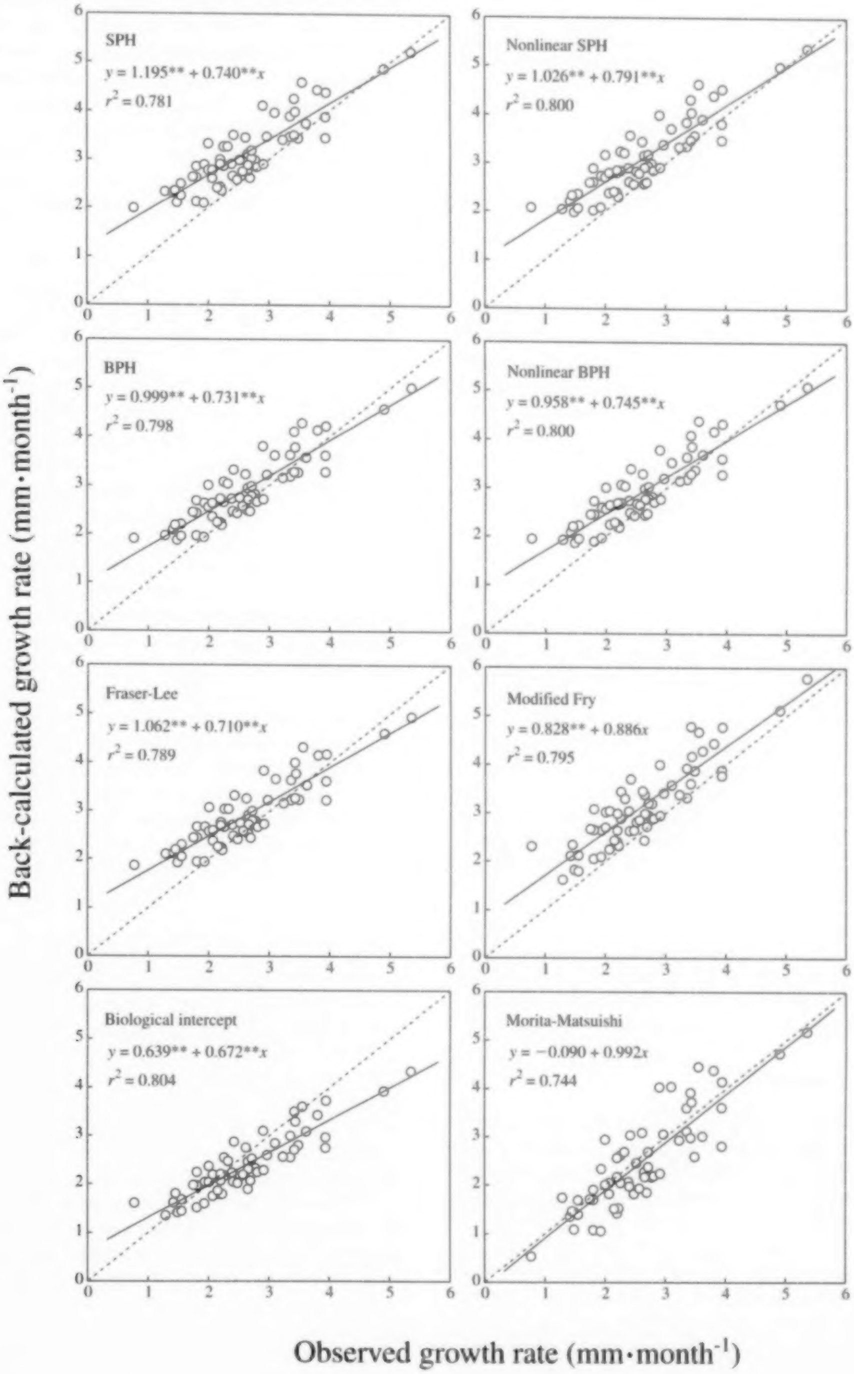


Fig. 3. Relationships between back-calculated growth rate and observed growth rate using SPH, BPH, Fraser–Lee, biological intercept, nonlinear SPH, nonlinear BPH, modified Fry, and Morita–Matsuishi models. Dashed lines, 1:1 relationships; solid lines, fitted regressions. Regression equations and r^2 values are given in each panel. Asterisks denote significant differences of the slopes and intercepts from 1 and 0, respectively, based on t tests ($*p < 0.01$; $**p < 0.001$).



Institute of Fisheries Science, Komaki 1088, Ueda, Nagano 386-0031, Japan, personal communication) that originated from his previous study (Yamamoto et al. 1997): age 0+ fish in June 1995, $L_0 = 34.6$ mm, $O_0 = 292$ µm, $n = 284$. As white-spotted char fry emerge from gravel in late May, the biological intercept nearly corresponds to size at emergence.

Highly significant relationships were found between otolith radius and fork length (Fig. 1). We obtained five regression equations:

$$(16)\quad O = 320 + 7.16L \ (r^2 = 0.917,\ n = 279)$$

$$(17)\quad L = -30.6 + 0.128O \ (r^2 = 0.917,\ n = 279)$$

$$(18)\quad \ln O = 3.45 + 0.758 \ln L \ (r^2 = 0.935,\ n = 279)$$

$$(19)\quad \ln L = -3.94 + 1.23 \ln O \ (r^2 = 0.935,\ n = 279)$$

$$(20)\quad L = L_0 - 0.000967 O_0^{1.62} + 0.000967\, O^{1.62} \ (n = 279)$$

where O is otolith radius (micrometres) and L is fork length (millimetres). However, multiple regression analysis identified significant effects of age on otolith radius in addition to fork length (age: $t = 15.7$, $p < 0.001$; fork length: $t = 24.5$, $p < 0.001$). We obtained the following multiple regression equation:

$$(21)\quad O = 357 + 4.60L + 11.5t \ (r^2 = 0.956,\ n = 279)$$

where t is age (months).

Because the otolith annulus (opaque) of white-spotted char forms from late May to July each year (Yamamoto et al. 1992), the end of the second annulus corresponded to the time when tagged fish were measured for fork length. We back-calculated the fork lengths in July 1998 (the end of the second annulus) of the 59 fish tagged in July 1998 and recaptured in October 1999 or 2000 using the eight back-calculation models (eqs. 5, 6, 7, 8, 9, 10, 13, and 15). Also, we calculated the absolute growth rates (millimetres per month) from July 1998 to the time of capture in October 1999 or 2000. As fork length in July 1998 was either observed from tagged fish or back-calculated from the otolith, we could obtain both observed and back-calculated growth rates for 59 tagged fish. Unfortunately, we could not use the TVG model (eq. 11) suggested by Sirois et al. (1998) because the parameter of the growth effect R was negligible and was not statistically significant (slope of the fork length – otolith radius relationship = 0.0950 – 0.0000302 × absolute growth rate, $r^2 = 0.003$, $n = 279$, $p = 0.394$).

In this study, we examined the relationships (*i*) between back-calculated fork lengths and observed fork lengths and (*ii*) between back-calculated growth rates and observed growth rates to see if the back-calculation models were accurate.

Results and discussion

The back-calculated fork lengths were highly correlated with the observed fork lengths irrespective of back-calculation model with $r^2 = 0.744$–0.839 (Fig. 2). However, the intercepts from the regressions of back-calculated fork lengths on observed fork lengths obtained from the biological intercept and modified Fry were significantly different from 0 (all $p < 0.001$); the slopes from the regressions obtained from nonlinear SPH, nonlinear BPH, biological intercept, and modified Fry were significantly different from 1 (all $p < 0.01$). Therefore, our results suggested that simple traditional models (SPH, BPH, and Fraser–Lee) and the new model gave the unbiased back-calculated fork length at the individual level. The back-calculated growth rates were also correlated with the observed growth rates irrespective of back-calculation model with $r^2 = 0.744$–0.804 (Fig. 3). However, back-calculated growth rates did not correspond well to observed growth rates except for the new model proposed here. For all previous models, the intercepts from the regressions of back-calculated growth rates on observed growth rates were significantly greater than 0 (all $p < 0.001$); the slopes from the regressions were significantly less than 1 ($p < 0.001$ except for modified Fry). These results suggested that the growth rates of slow-growing fish were overestimated, as we expected. As the otolith increases in size substantially even though somatic growth is little (age effect or growth effect), the previous models would overestimate the past growth rates of slow-growing fish. Our model incorporating age effect did not show such bias.

Many studies of various species support the assumption that otoliths increase in size continuously without somatic growth (e.g., striped bass (*Morone saxatilis*), red seabream (*Pagrus major*), and spot (*Leiostomus xanthurus*): Secor et al. 1989; Atlantic salmon (*Salmo salar*): Wright et al. 1990; goldfish (*Carassius auratus*): Mugiya 1990; greenback flounder (*Rhombosolea tapirina*): Jenkins et al. 1993; American eel (*Anguilla anguilla*): Holmgren 1996; whiting (*Sillaginodes punctata*): Barber and Jenkins 2001). A physiological study showed that calcium deposition on otoliths of rainbow trout (*Oncorhynchus mykiss*) does not stop during starvation, although deposition rate is reduced (Mugiya and Oka 1991). Furthermore, many empirical studies showed that older fish have larger otoliths than equal-sized younger fish (e.g., Pawson 1990; Fletcher 1991; Francis et al. 1993), suggesting that the age effect is apparent, as found in the results of this study (Fig. 1*b*). Secor and Dean (1992) and Hare and Cowen (1995) argued that incorporating age dramatically improves the regression between fish body length and otolith length, such that age should be included in regression back-calculation formulae to account for either the growth effect or the age effect. Nevertheless, few studies incorporate the age effect into the back-calculation model, or rather, most previous studies persisted in the simple coupling of otolith and somatic growth. To our knowledge, this study is the first attempt to incorporate the age effect into a general back-calculation model.

The back-calculation model proposed here should be useful for estimating past growth rates to reduce bias, at least at the individual level. As age is determined in many cases when a back-calculation is made, our model does not impose additional data requirements and is easy to apply. A notable feature of our model is that it can back-calculate fish length during zero or negative growth periods. Therefore, our model may be particularly suitable for long-lived, slow-growing fish. Unfortunately, it failed to increase the correlation coefficient between back-calculated and observed length but rather decreased it a little compared with previous models, even though the number of model parameters increased. However, we avoid bias associated with growth rates. Incorporating the age effect into back-calculation models merits further study.

Acknowledgments

We thank Y. Kanno, K. Nishimura, and S.H. Morita for their invaluable advice and encouragement during the study,

S. Yamamoto for providing biological intercept data and help in the field, and Y. Takashima for technical support with image analysis and help in the field. We also thank two anonymous referees for improving the paper. This work was partially supported by a grant-in-aid for Research Fellow of the Japan Society for the Promotion of Science (No. 07054) to K. Morita from the Japanese Ministry of Education, Culture, Sports, Science and Technology.

References

Barber, M.C., and Jenkins, G.P. 2001. Differential effects of food and temperature lead to decoupling of short-term otolith and somatic growth rates in juvenile King George whiting. J. Fish Biol. **58**: 1320–1330.

Campana, S.E. 1990. How reliable are growth back-calculations based on otoliths? Can. J. Fish. Aquat. Sci. **47**: 2219–2227.

Fletcher, W.J. 1991. A test of the relationship between otolith weight and age for the pilchard *Sardinops neopilchardus*. Can. J. Fish. Aquat. Sci. **48**: 35–38.

Francis, M.P., Williams, M.W., Pryce, A.C., Pollard, S., and Scott, S.G. 1993. Uncoupling of otolith and somatic growth in *Pagrus auratus* (Sparidae). Fish. Bull. (Washington, D.C.), **91**: 159–164.

Francis, R.I.C.C. 1990. Back-calculation of fish length: a critical review. J. Fish Biol. **36**: 883–902.

Fukuwaka, M., and Kaeriyama, M. 1997. Scale analyses to estimate somatic growth in sockeye salmon, *Oncorhynchus nerka*. Can. J. Fish. Aquat. Sci. **54**: 631–636.

Hare, J.A., and Cowen, R.K. 1995. Effect of age, growth rate, and ontogeny on the otolith size – fish size relationship in bluefish, *Pomatomus saltatrix*, and the implications for back-calculation of size in fish early life history stages. Can. J. Fish. Aquat. Sci. **52**: 1909–1922.

Holmgren, K. 1996. Otolith growth scaling of the eel, *Anguilla anguilla* (L.), and back-calculation errors revealed from alizarine labeled otoliths. Nord. J. Freshw. Res. **72**: 71–79.

Horppila, J., and Nyberg, K. 1999. The validity of different methods in the backcalculation of the lengths of roach — a comparison between scales and cleithra. J. Fish Biol. **54**: 489–498.

Jenkins, G.P., Shaw, M., and Stewart, B.D. 1993. Spatial variation in food-limited growth of juvenile greenback flounder, *Rhombosolea tapirina*: evidence from otolith daily increments and otolith scaling. Can. J. Fish. Aquat. Sci. **50**: 2558–2567.

Klumb, R.A., Bozek, M.A., and Frie, R.V. 2001. Validation of three back-calculation models by using multiple oxytetracycline marks formed in the otoliths and scales of bluegill × green sunfish hybrids. Can. J. Fish. Aquat. Sci. **58**: 352–364.

Morita, K., and Yamamoto, S. 2001. Contrasts in movement behaviour of juvenile white-spotted charr between stocks above and below a dam. Fish. Sci. **67**: 179–181.

Morita, K., Yamamoto, S., and Hoshino, N. 2000. Extreme life history change of white-spotted char (*Salvelinus leucomaenis*) after damming. Can. J. Fish. Aquat. Sci. **57**: 1300–1306.

Mugiya, Y. 1990. Long-term effects of hypophysectomy on the growth and calcification of otoliths and scales in the gold fish, *Carassius auratus*. Zool. Sci. **7**: 273–279.

Mugiya, Y., and Oka, H. 1991. Biochemical relationship between otolith and somatic growth in the rainbow trout *Oncorhynchus mykiss*: consequence of starvation, resumed feeding, and diel variations. Fish. Bull. (Washington, D.C.), **89**: 239–245.

Pawson, M.G. 1990. Using otolith weight to age fish. J. Fish Biol. **36**: 521–531.

Reznick, D., Lindbeck, E., and Bryga, H. 1989. Slower growth results in larger otoliths: an experimental test with guppies (*Poecilia reticulata*). Can. J. Fish. Aquat. Sci. **46**: 108–112.

Secor, D.H., and Dean, J.M. 1992. Comparison of otolith-based back-calculation methods to determine individual growth histories of larval striped bass, *Morone saxatilis*. Can. J. Fish. Aquat. Sci. **49**: 1439–1454.

Secor, D.H., Dean, J.M., and Baldevarona, R.B. 1989. Comparison of otolith growth and somatic growth in larval and juvenile fishes based on otolith/fish length relationships. Rapp. P.-v. Réun. Cons. Int. Explor. Mer, **191**: 431–438.

Sirois, P., Lecomte, F., and Dodson, J.J. 1998. An otolith-based back-calculation method to account for time-varying growth rate in rainbow smelt (*Osmerus mordax*) larvae. Can. J. Fish. Aquat. Sci. **55**: 2662–2671.

Smedstad, O.M., and Holm, J.C. 1996. Validation of back-calculation formulae for cod otoliths. J. Fish Biol. **49**: 973–985.

Suthers, I.M., and Sundby, S. 1996. Role of the midnight sun: comparative growth of pelagic juvenile cod (*Gadus morhua*) from the Arcto-Norwegian and a Nova Scotian stock. ICES J. Mar. Sci. **53**: 827–836.

Vigliola, L., Harmelin-Vivien, M., and Meekan, M.G. 2000. Comparison of techniques of back-calculation of growth and settlement marks from the otoliths of three species of *Diplodus* from the Mediterranean Sea. Can. J. Fish. Aquat. Sci. **57**: 1291–1299.

Wright, P.J., Metcalfe, N.B., and Thorpe, J.E. 1990. Otolith and somatic growth rates in Atlantic salmon parr, *Salmo salar* L: evidence against coupling. J. Fish Biol. **36**: 241–249.

Yamamoto, S., Nakano, S., and Tokuda, Y. 1992. Variation and divergence of the life-history of Japanese charr *Salvelinus leucomaenis* in an artificial lake – inlet stream system. [In Japanese with English summary.] Jpn. J. Ecol. **42**: 149–157.

Yamamoto, S., Shinomi, H., and Goto, A. 1997. The effect of birth date on growth of juvenile white-spotted charr *Salvelinus leucomaenis*. Fish. Sci. **63**: 931–933.

Zenitani, H. 1999. Size-specific changes in amount of lipid daily growth rate of early larval sardine, *Sardinops melanostictus*, in the main Kuroshio Current and its offshore waters off eastern Japan. Mar. Biol. **134**: 645–652.

Appendix. Derivation of the new back-calculation model

If we assume otolith growth as in eq. 14, the otolith length of the *i*th individual fish is described by

$$\text{(A1)}\quad O_{T,i} = (\alpha + \beta L_{T,i} + \gamma T)\varepsilon_i$$

where $O_{T,i}$ is the otolith length of the *i*th fish at the time of capture T, $L_{T,i}$ is the body length of the *i*th fish at the time of capture T, and ε_i is the proportional deviation of the otolith length of the *i*th fish from the average. The back-calculated body length of the *i*th fish at age t ($L_{t,i}$) is

$$\text{(A2)}\quad L_{t,i} = \frac{1}{\beta}\left(\frac{O_{t,i}}{\varepsilon_i} - \alpha - \gamma t\right)$$

where $O_{t,i}$ is the otolith length of the *i*th fish at annulus (age) t. Thus, we obtain eq. 15 by substituting ε_i of eq. A1 into eq. A2.

Parentage and grandparentage assignment with known and unknown matings: application to Connecticut River Atlantic salmon restoration

Benjamin H. Letcher and Tim L. King

Abstract: Large-scale use of parentage assignment of stocked Connecticut River, U.S.A., Atlantic salmon (*Salmo salar*) fry to determine stocking tributary of emigrating smolts and returning adults requires both parentage assignment and grandparentage assignment. Using computer simulations, we explored the levels of genetic diversity necessary to provide adequate parentage and grandparentage assignment. When matings were known, as in a hatchery, 95% correct grandparentage assignment typically required at least twofold more alleles per locus than did 95% correct parentage assignment. Parentage assignment with unknown matings (as in the wild when parental genotypes can be assayed) required an intermediate number of alleles per locus and grandparentage assignment with unknown matings required the greatest genetic diversity. Computer simulations using empirical parental genotypes (14 loci) and known P1 matings indicated that the genetic diversity of Connecticut River Atlantic salmon broodstock is sufficient to assign all F1 and 97% of F2 offspring to the correct set of two parents or four grandparents, respectively. Grandparentage assignment with unknown P1 matings was ineffective (assignment success = 20%). These results indicate that the combination of parentage and grandparentage assignment can be used as a non-lethal, permanent, heritable "mark" to identify stocking tributary or other management directed characteristics of stocked fry.

Résumé : La détermination à grande échelle de la filiation chez des alevins du Saumon de l'Atlantique (*Salmo salar*) ensemencés dans le fleuve Connecticut, États-Unis, dans le but de reconnaître le tributaire d'ensemencement des saumoneaux qui émigrent et des adultes qui reviennent, requiert l'identification des parents et des grands-parents. Des simulations à l'ordinateur nous ont permis d'explorer les niveaux de diversité génétique nécessaires à l'identification adéquate des parents et des grands-parents. Lorsque les accouplements sont connus, par exemple dans une pisciculture, la détermination des grands-parents avec un niveau d'exactitude de 95% requiert normalement au moins deux fois plus d'allèles à un locus que la détermination des parents avec la même exactitude. La détermination de la filiation lorsque les accouplements sont inconnus (comme en nature lorsque les génotypes parentaux peuvent être analysés) requiert un nombre intermédiaire d'allèles par locus et la détermination des grands parents lors d'accouplements inconnus exige la plus grande diversité génétique. Des simulations qui utilisent des génotypes parentaux empiriques (14 locus) et des accouplements P1 connus indiquent que la diversité génétique du stock des reproducteurs du Saumon de l'Atlantique du fleuve Connecticut est suffisamment grande pour permettre l'identification précise des deux parents de toute la F1 et des quatre grands-parents de 97% de la F2. La détermination des grands-parents lorsque les accouplements de la P1 sont inconnus est inefficace (succès des détermination = 20%). Ces résultats démontrent que la détermination combinée des parents et des grands-parents peut servir de « marqueur » non-létal, permanent et héréditaire pour identifier le tributaire d'ensemencement et d'autres caractéristiques des alevins ensemencés qui sont importantes pour la gestion.

[Traduit par la Rédaction]

Introduction

Overharvest and habitat degradation have necessitated extensive conservation plans for many fish species in an attempt to maintain or reestablish population viability. Migratory salmonids, in particular, require detailed management plans because of their complex life histories. Anadromous salmonids depend on freshwater habitat for spawning and juvenile rearing, riverine habitat for migration pathways, and oceanic habitat for growth to spawning size. Human impact on these habitats and on the connections between them has caused numerous populations to decline in recent years, to the point that many are severely depleted or extinct (Allendorf et al. 1997; McDowall 1999). Often, hatchery-based stocking programs have been implemented to supplement depleted runs or to reintroduce and maintain previously extirpated populations (Kennedy 1988; Moring et al. 1995; Schramm and Piper 1995). Although hatchery-stocked fish may boost fish runs, there are concerns about introgression of artificially selected hatchery stock into natural stocks (Bernatchez and Wilson 1999), genetic differences

Received September 22, 2000. Accepted July 4, 2001. Published on the NRC Research Press Web site at http://cjfas.nrc.ca on August 22, 2001.
J15991

B.H. Letcher.[1] Biological Resources Division, U.S. Geological Survey, S.O. Conte Anadromous Fish Research Center, One Migratory Way, P.O. Box 796, Turners Falls, MA 01376, U.S.A.
T.L. King. Biological Resources Division, U.S. Geological Survey, Aquatic Ecology Laboratory, 1700 Leetown Road, Kearneysville, WV 25430, U.S.A.

[1] Corresponding author (e-mail: bletcher@forwild.umass.edu).

Can. J. Fish. Aquat. Sci. **58**: 1812–1821 (2001) DOI: 10.1139/cjfas-58-9-1812

between wild and hatchery stocks (Waples 1991), and tracking the performance of cultured fish in the wild (Fleming et al. 2000).

Atlantic salmon (*Salmo salar*) were extirpated from the southernmost extent of their North American range (Connecticut River, U.S.A.) nearly 150 years ago. An extensive restoration effort has been underway for the last 30 years. The current source of hatchery broodstock for the restoration effort is adult sea-run returns that are trapped at hydroelectric facilities. Most fish are retained for broodstock, which essentially eliminates natural reproduction of wild fish. As most sea-run adults are not allowed to return to the sites in which they were stocked as fry, tributary-specific information on the production of adults returning to the river is not available. Unlike Pacific salmon, which are often stocked as parr or smolts and can therefore be tagged before stocking (Achord et al. 1996), the small size (25 mm) of stocked Atlantic salmon fry precludes use of physical tags for identifying stocking tributaries. Population sizes are insufficient for tags or marks, such as otolith banding (Volk et al. 1994; Schroder et al. 1995) or coded wire tags that require lethal sampling (Ballou et al. 1995; Norris et al. 2000).

Our aim is to describe the use of broodstock multilocus genotypes as the basis of parentage assignment (Chakraborty et al. 1988; Marshall et al. 1998; Bernatchez and Duchesne 2000) in an active restoration effort. Two characteristics in particular make the Connecticut River Atlantic salmon restoration effort and the application of genetic tools to the restoration somewhat unique. First, most of the stocked offspring (~75%, F2 fry) derive from crosses among F1 domestic broodstock and the remaining 25% are F1 fry. The domestic broodstock and F1 fry are the offspring of sea-run returns (P1). Identification of stocked offspring via parentage analysis from both P1 and F1 broodstock requires either (*i*) tagging and genotyping all F1 broodstock (3000 fish) in addition to the P1 broodstock (150–500 fish) or (*ii*) tracking matings of family-tagged F1 broodstock and identifying families of F2 offspring through grandparentage analysis. Owing to the expense of genotyping 3000 F1 broodstock for each year-class of fish, we have chosen to identify families of F2 stocked fry based on their common grandparents.

Second, because most adults are not allowed to return to natal tributaries, natural reproduction is negligible. Thus a large measure of control exists over both the mating of all fish in the system and in the stocking location of offspring. This level of control provides the opportunity to test hypotheses about survival of fish from different stocking locations without concerns about interfering with naturally spawning fish.

We describe an approach that takes advantage of the inherent genetic differences among individual fish to use parentage assignment of stocked offspring as an internal, nondestructive tag to identify released animals and evaluate their growth, movement, and (or) survival rates. Norris et al. (2000) described a similar approach in an aquaculture system. Parentage assignment has been used successfully with Atlantic salmon (O'Reilly et al. 1998; Herbinger et al. 1999; Martinez et al. 2000) and a variety of other aquatic species (Wolfus et al. 1997; Garcia de Leon et al. 1998; Volckaert and Hellemans 1999). Researchers have estimated levels of genetic diversity required for effective parentage analysis using either actual (Estoup et al. 1998) or simulated (Bernatchez and Duchesne 2000) allele value data. Neff et al. (2000) have developed parentage assignment algorithms and statistical confidence estimates for incomplete sampling of candidate parents. We present an analysis using both actual and simulated allele data for complete sampling of candidate parents and grandparents.

In addition to parentage analysis with known matings in a hatchery, researchers often determine parentage for fish in the wild. A common scenario with salmonids is the sampling of adults at a weir as they ascend to spawning areas. Fish are released above the weir after sampling and are allowed to spawn naturally. Their offspring are sampled at a later date to determine parentage. Parentage assignment in the wild has been used to estimate effective population size, the relative success of alternative mating tactics, the interaction between hatchery and wild fish, the specific traits associated with reproductive success, and the heritability of specific traits (summarized by Blanchfield and Jones 2001. To extend our analysis to a natural situation and to provide a comparison between controlled situations with known matings and uncontrolled situations where adult genotypes are determined but mating pairs are unknown, we also determined the level of genetic diversity required for successful parentage assignment with unknown matings and compared this to the level required with known matings. This comparison also provides information on the necessity of tracking individual matings in a hatchery over a wide range of genetic diversity levels.

Materials and methods

Tissue collection

Adult samples consisted of fin clips taken from returning sea-run Atlantic salmon (1997) held at the Richard Cronin National Fish Hatchery (U.S. Fish and Wildlife Service (USFWS)) in Sunderland, Mass., U.S.A., or at the State of Connecticut Whittemore Atlantic salmon holding facility, Barkhamsted, Conn., U.S.A. We also took fin clips from juveniles with known parentage reared at the White River National Fish Hatchery (USFWS), Bethel, Vt., U.S.A. Anal fin clips were preserved in 95% ethanol and stored on ice until fixation for at least 24 h.

DNA extraction and isolation

Genomic DNA was isolated from fin tissue using the PureGene DNA extraction kit (Gentra Systems, Minneapolis, Minn., U.S.A.). DNA quantity was determined by fluorescence assay (Labarca and Paigen 1980) and the quality was visually inspected in 1% agarose gels. Fourteen microsatellite loci were screened in all fish (SSOSL20, SSOSL85, and SSOSL417 (Slettan et al. 1995, 1996); Ssa85, Ssa171, Ssa197, and Ssa202 (O'Reilly et al. 1996); SSLEEI84, SSLEEL53, SSLEEN82, and SSLEER15, GenBank accession numbers U86703, U86704, U86706, and U86708, respectively (W.B. Schill, U.S. Geological Survey, Biological Resources Division, Leetown Science Center, Leetown, W. Va., U.S.A., unpublished data); Sfo23 (Angers et al. 1995); and SsaF43 and SsaD30 (J.A. Sanchez Prado, Universidad de Oviedo, Oviedo, Spain, personal communication). Each PCR consisted of 6 ng$\cdot\mu$L^{-1} of genomic DNA, 1× PCR buffer (10 mM Tris–HCl, pH 8.3, 50 mM KCl), 2 mM $MgCl_2$, 0.2 mM dNTPs, 0.3 µM of each primer (forward primer fluorescently labeled with TET, FAM, or HEX; Applied Biosystems, Foster City, Calif., U.S.A.), 1.0 unit of Taq polymerase (Applied Biosystems), and deionized water added to achieve the selected final volume. The amplification cycle consisted of an initial denaturing at 94°C for 2 min followed by 35 cycles of 94°C denaturing for 30 s, 55°C or 58°C annealing for 30 s, and 72°C extension for 30 s. Cycling culminated with a 5-min extension at 72°C. Thermal cycling was performed in an MJ DNA Engine PTC 200 (MJ Research, Watertown, Mass.). Generally, 1 μL of PCR product was diluted 3:1 with deionized water and thoroughly mixed. This dilution (1 μL) was added to 12 μL of deionized formamide and 0.5 μL of the internal size standard GeneScan-500 (Applied Biosystems). The size standard contained DNA fragments fluorescently labeled with the dye phosphoramidite TAMRA (red). This PCR product–size standard–formamide mixture was heat denatured at 94°C for 2 min and placed immediately on ice for at least 5 min. The mixture was then subjected to capillary electrophoresis on an ABI PRISM 310 Genetic Analyzer (Applied Biosystems). We used Applied Biosystems GeneScan software and Genotyper v. 2.0 DNA fragment analysis software to generate genotype data and to score, bin, and output allelic (and genotypic) designations for each salmon. GENEPOP version 3.1 (Raymond and Rousset 1995) was used to test for conformance to Hardy–Weinberg (H–W) expectations and to test for the presence of genotypic linkage disequilibrium at each locus.

Parentage assignment

Simulated parental and simulated offspring data

We developed a computer simulation (Visual Basic, available from corresponding author) to explore how variation in levels of genetic diversity (alleles per locus, number of loci) influenced assignment success for cases of known and unknown matings. The simulation used a maximum likelihood approach (SanCristobal and Chevalet 1997; Bernatchez and Duchesne 2000) to determine parentage by searching the matings for pairs of parents that could have created each offspring's genotype. Each parental pair was assigned a likelihood score and all scores were ranked. The pair with the highest likelihood score was labeled the most likely pair and was compared to the actual pair for that offspring. If the most likely pair equaled the actual pair, the assignment was scored as correct, otherwise it was incorrect. With known matings, the set of candidate pairs (pairs for which parentage was tested) included only the pairs of mated fish. This contrasts with searching over all possible parent pairs when matings were unknown.

At the beginning of each simulation run, alleles were assigned randomly to each adult (based on a normal allele distribution, coefficient of variation (CV) = 1). Matings were also random and adults mated only once. Offspring were assigned alleles based on Mendelian inheritance. Input to the model included a series of variables (described below) and output included whether each offspring was assigned to the correct family or an incorrect family. Assignment success for a population of offspring equaled the proportion of correct assignments.

Input variables were number of loci examined, number of possible alleles per locus, number of parents, number of offspring per parent, and whether matings were known or unknown. Alleles were assigned to parents with a normal distribution as the integer of $\text{numA}/2 \cdot N(0,1) \cdot \text{CV} + \text{numA}/2$, where numA was the number of alleles per locus and $N(0,1)$ designated a normal distribution with mean = 0 and standard deviation = 1. We varied systematically values of the input variables (number of loci, 2 to 16; number of alleles per locus, 3 to 20) to define the assignment success parameter space. For these simulations, we did not vary number of parents (100) or number of offspring per parent (10). In a separate set of simulations, we also examined the effects of the number of parents on assignment success by varying parent number from 50 to 10 000 using 14 loci, 12 alleles per locus, and a normal allele distribution.

Actual parental and simulated offspring data

Using a normal allele distribution as in the previous section is useful for assessing general patterns of assignment success and for providing guidelines for populations with unknown distributions, but actual allele distributions for a population of fish rarely fit simple statistical distributions well. To test assignment success using actual parental data, we incorporated allele values of individual sea-run return broodfish from the Connecticut River Atlantic salmon restoration program (Fig. 1). The actual parental data simulations were identical with those in the previous section, except that allele values from individual broodfish were assigned to parents instead of values drawn randomly from the statistical distribution. The number of parents was 169, the number of offspring per parental pair was 10, and we tested both known and unknown matings. We did not assign sex to individual broodstock. Using actual parental and simulated offspring data with known matings represents a situation where matings are tracked in a hatchery or, in a rare case, where all matings are known in the wild. Actual parental data with unknown matings represents a situation where genotypes of the potential parents are known but actual mating pairs are unknown, i.e., broodstock in a hatchery where matings are not tracked or when adult salmon are sampled at a counting fence and released above to spawn.

Actual parental and actual offspring data

To test for Mendelian inheritance of alleles and our ability to successfully identify actual parents, we examined eight loci (Ssa85, SSOSL85, SSLEEN53, Ssa197, Ssa202, SSLEEN82, SSOSL417, Ssa171) from offspring with known parents and parental allele values. Offspring from different families were reared separately in the hatchery. We took fin clips from offspring once they were 25 mm long and tested parentage assignment for five offspring from each of eight families. For each offspring, we assessed parentage for every possible combination of all potential parents (169 parents for a total of 14 196 combinations) assuming matings were unknown, or for the subset of actual matings (16 parents, eight family combinations) assuming matings were known.

Effect of error

We examined the effect of genotyping error on assignment success with both known and unknown matings by varying the error rate from 0 to 0.05. We used the actual parental broodstock data for parental allele values and allowed error in the offspring allele values. We introduced error in allele values by simply adding a value of two (dinucleotides) or four (tetranucleotides) to the correct allele value of any allele that was assigned an error. This error structure represents the most common type of error deriving from genotyping or mutation (deletions of two or four are also possible, but we modeled additions only for simplicity). All alleles had an equal probability of error equal to the error rate. Errors were assigned on an allele-by-allele basis at each locus so that an individual offspring could have errors at more than one locus or even, in an extreme case, in each allele within a locus.

Effect of variation in parent number

We examined the interacting effects of varying parent number (50, 110, 210, 310, 410), number of loci (4–16), and number of alleles (4–20) on assignment success with unknown matings by running three replicates of all combinations of variables. We report the minimum number of alleles required to reach 95% assignment success in each combination.

Grandparentage assignment

Simulated data

Most (~75%) of the Atlantic salmon fry (F2) stocked into the Connecticut River are offspring of domestic broodstock (F1) that

Fig. 1. Allele frequency distributions for 14 loci from Connecticut River Atlantic salmon sea-run returns in 1997. *N* is the number of alleles per locus.

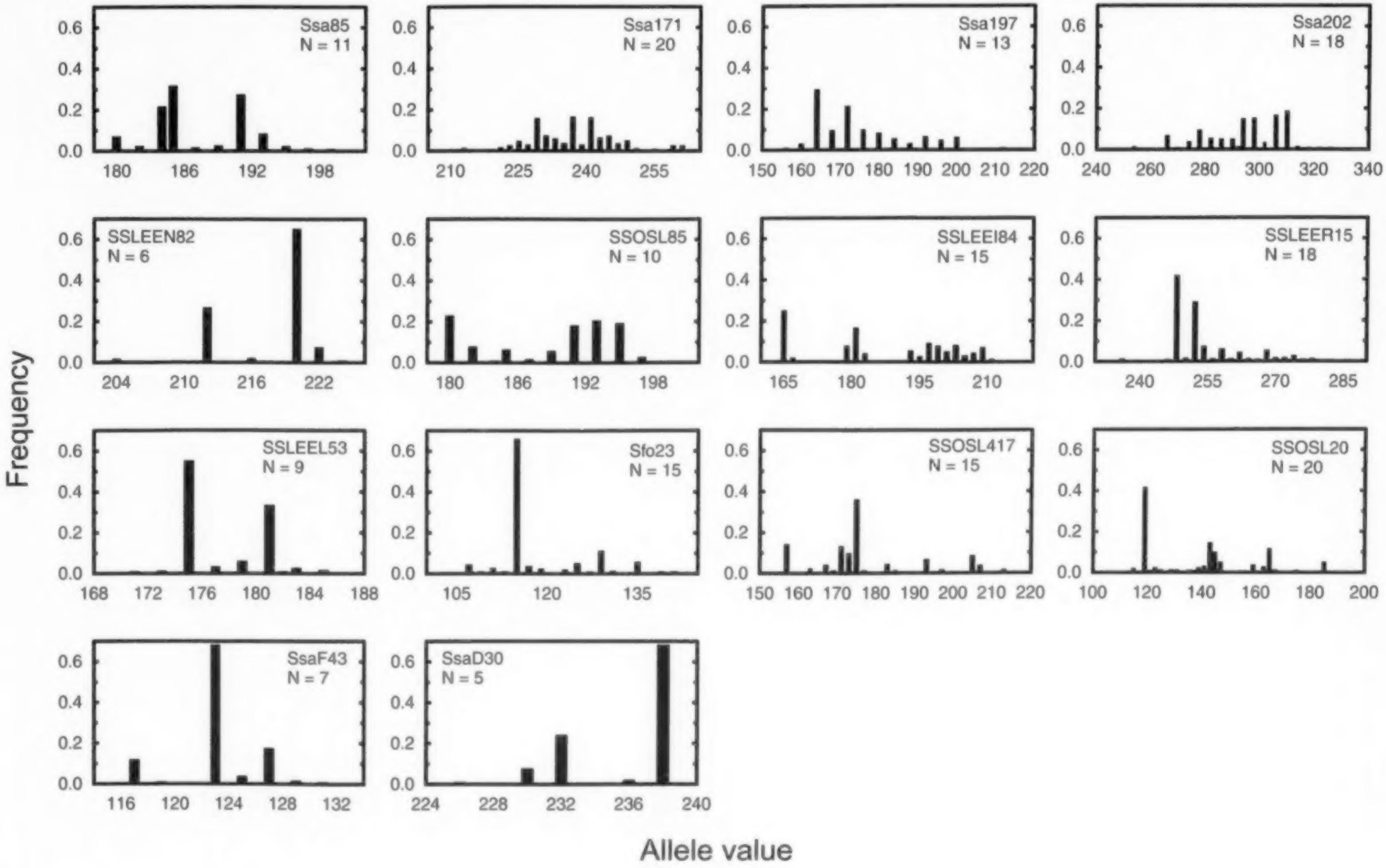
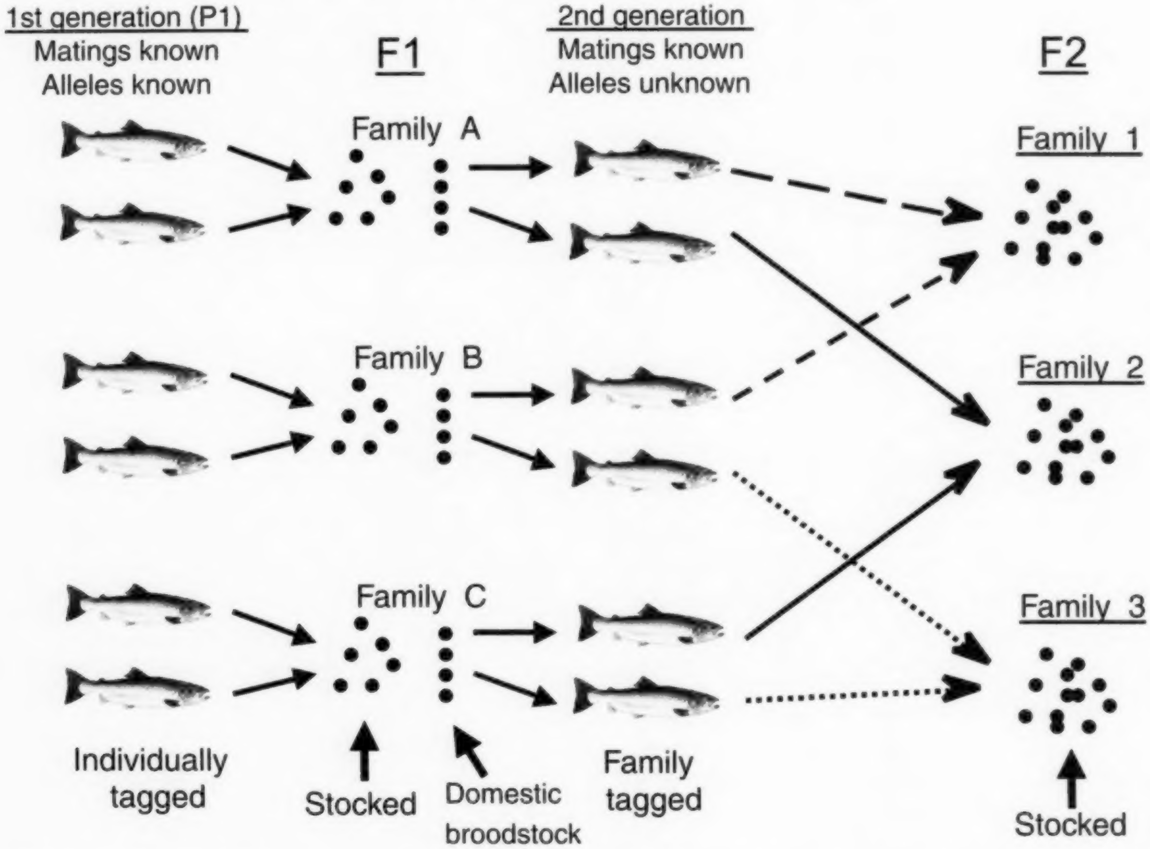


Fig. 2. Representation of the mating scheme for implementing grandparentage assignment in a fish hatchery. Adult Atlantic salmon returning to the river are captured and transferred to the hatchery where they are individually tagged and sampled for genotype. Matings between closely related individuals are avoided and most of the offspring are stocked into rivers. A portion of the offspring is retained for F1 broodstock and is tagged according to family. Matings among fish from the F1 families produce F2 offspring that are all stocked into rivers. In a given year, 25% of the stocked fish are F1 offspring and 75% are F2 offspring.



are first-generation progeny of sea-run returns (P1). Thus, the majority of stocked fry are second-generation offspring of sea-run returns. The number of first-generation domestic broodstock (F1, >3000) is too large for routine genotyping that could be used to determine parentage of their (second generation) stocked offspring as in the parentage assignment section. To explore the possibility of identifying grandparents of the F2 fry, we also designed the simulation to determine grandparentage assignment.

The scenario we tested with the two-generation simulation was as follows (Fig. 2). All sea-run returns (P1) are tagged and genetic samples are analyzed (in late spring). During spawning in the hatchery (late fall), each mating is recorded and creates a family (4000–8000 offspring with a single mother and father). Over 90% of the offspring from each family are stocked as first-generation fry (F1). The rest are tagged with passive integrated transponder (PIT) tags to identify family and are raised to spawning size at the hatchery. On reaching spawning size (2–3 years), fish from different families within an age-class are mated creating F1 pairs and the offspring of the F1 pairs are stocked as fry (F2); thus, we know the multilocus genotypes of the sea-run adult returns (P1) and we know the family of each individually tagged first-generation domestic broodstock.

To assess the success of assignments under this scenario, we ran the simulation using the same input variables as in the parent assignment section but generated both F1 and F2 offspring. F1 offspring were generated as above and F2 offspring were created from random matings between the F1's. Matings between fish from the same F1 family were not allowed (in practice and in the simulations). For each offspring, the simulation searched through either all possible combinations of P1 parent pairs (unknown matings) or through the set of P1 parental pairs that were actually mated (known matings). Sets of grandparents were evaluated by an extension of the maximum likelihood approach for parentage assignment (SanCristobal and Chevalet 1997) to grandparentage assignment (Appendix). For both known and unknown P1 matings, we report the proportion of F2 offspring for which the correct set of four grandparents was found for combinations of the two input variables (alleles per locus, number of loci). Running the simulation for all F2 offspring took considerable computer time (approximately 12 min per offspring on a 1.5 GHz computer). Based on initial runs, assignment success values converged with 25 F2 offspring, so assignment was tested on every 100th offspring out of the 2500 total.

Actual parental and simulated offspring data

In addition to smooth probability distributions, we also ran the grandparentage assignment simulation using raw allele value data from the 1997 broodstock (Fig. 1) for the initial distribution of parental (P1) alleles. Sets of grandparents were searched for assuming both known and unknown mating of P1 fish.

Results

Connecticut River genetic diversity

Multilocus genotypes at 14 microsatellite DNA loci were determined for 169 sea-run Atlantic salmon returning to the Connecticut River in 1997. Considerable genetic diversity was observed as loci yielded between five (SsaD30) and 20 (Ssa171 and SSOSL20) alleles with a total of 182 distinct alleles (Fig. 1). The mean (standard error (SE)) sample size per locus was 158.1 (1.6, occasional allele values at specific loci were not determined) and the mean (SE) number of alleles per locus was 13.0 (1.4). The mean (SE) direct count heterozygosity for all loci was 0.68 (0.053). When each locus was analyzed separately for significant deviations from expected H–W frequencies of genotypes, five (36%) were found to have p values significant at the overall 0.05 level after correcting for the number of tests ($p = 0.0036$). Of the five deviations observed, four (SSLEEI84, SSOSL20, SsaF43, Sfo23) were because of heterozygote deficiencies, and one (SSLEEL53) showed heterozygote excesses. These deviations could have resulted from nonrandom mating in the hatchery system, mixing of year classes, admixture from salmon straying from other rivers, genotyping errors, and (or) the presence of null alleles. Although we cannot determine the cause of these deviations at the present time, we have not observed null alleles among several families surveyed initially to determine the utility of these markers (T.L. King, unpublished data). Significant genotypic linkage disequilibrium was identified in three of 91 (3.3%) comparisons within the 1997 sea-run population. This negligible amount of linkage disequilibrium could result from the admixture of year-classes combined with small effective population sizes (and (or) sampling siblings within year-classes). Regardless, this level of disequilibrium indicates that physical linkage of loci appears unlikely.

Parentage assignment

Simulated parental and simulated offspring data

Effective parentage assignment with unknown matings required considerably more alleles per locus for a particular number of loci than did parentage assignment with known matings (Table 1). An assignment success of 95% was possible with 12 or fewer alleles per locus even with two or four loci when matings were known, but this level of assignment success was not possible with unknown matings. With known matings, greater than 95% correct parentage assignment required fewer than three alleles per locus for eight or more loci, whereas 95% correct assignment with unknown matings required four or more alleles across the range of numbers of loci examined (2–16, Table 1). Assignment success was 100% for as many as 10 000 initial parents with known matings, 14 loci, and 12 alleles per locus.

Actual parental and simulated offspring data

Using parental allele values from the 1997 Connecticut River sea-run returns (14 loci, Fig. 1), assignment success equaled 100% with known and 99% with unknown matings.

Actual parental and actual offspring data

Assuming known matings, all 40 offspring were assigned (eight loci) to the correct parent pair. With unknown matings, two offspring were assigned incorrectly (95% assignment success). In one case, likelihood values were identical for the correct and incorrect pair. In the other, one parent was identified correctly, but the other parent was incorrect.

Effect of error

The effect of genotyping error on assignment success was similar between known and unknown matings. Increasing error in offspring allele values reduced assignment success exponentially:

$$\text{success} = 100.59 \cdot e^{-27.21 \cdot \text{error}}, \text{ unknown matings}$$

$$\text{success} = 99.8 \cdot e^{-27.57 \cdot \text{error}}, \text{ known matings}$$

($r^2 = 0.99$; $N = 60$ for each). Without error, assignment success was high (100%) but decreased to values below 30% with an error rate of 0.05. Assignment success was 50% with an error rate of 0.025. Assignment success of at least 90% required an error rate below 0.004, 95% below 0.002, and 99% below 0.00059.

Effect of variation in parent number

Increasing parent number reduced assignment success when numbers of loci were low but had less of an effect when there were more than eight loci (Table 2). With eight or fewer loci, twofold more alleles were required for >95% assignment success with 410 compared with 10 parents. Six or more alleles were sufficient to achieve 95% success with 10 to 16 loci up to 410 parents.

Grandparentage assignment

Simulated data

With both known and unknown matings, grandparentage assignment required at least twofold more alleles per locus than did parentage assignment for similar success levels (Table 1). The difference between the numbers of alleles required for similar levels of parentage and grandparentage assignment success with known matings was greatest with lower numbers of loci (<10). For example, with known matings and six loci, 95% assignment success required four alleles for parentage assignment but needed 14 for grandparentage assignment, whereas minimum allele numbers were <3 (parentage) and 6 (grandparentage) with 16 loci. In addition, for parentage assignment with known matings, higher levels of assignment success did not require more alleles per locus with eight or more loci, but through 16 loci, higher assignment success consistently required more alleles per locus for grandparentage assignment.

As with parentage assignment, grandparentage assignment required considerably more alleles per locus with unknown matings than with known matings (Table 1). With unknown matings, 95% assignment success was possible with fewer

Table 1. Minimum number of alleles per locus required for an assignment success of 50, 80, or 95% in the first or second generation with either known or unknown matings of the P1 parents.

Number of loci	Parentage assignment						Grandparentage assignment					
	Known matings, %			Unknown matings, %			Known matings, %			Unknown matings, %		
	50	80	95	50	80	95	50	80	95	50	80	95
2	6	8	12	>20	>20	>20	>20	>20	>20	>20	>20	>20
4	3	4	5	7	10	15	>20	13	17	>20	>20	>20
6	<3	<3	4	5	6	8	9	11	14	>20	>20	>20
8	<3	<3	<3	4	5	6	7	9	11	19	>20	>20
10	<3	<3	<3	3	4	5	6	7	8	13	>20	>20
12	<3	<3	<3	3	4	4	5	6	7	11	17	>20
14	<3	<3	<3	<3	3	4	5	6	7	10	13	20
16	<3	<3	<3	<3	<3	4	5	5	6	8	12	18

Note: Data are from simulated parent and simulated offspring computer runs with a normal parental allele distribution at each locus.

than 20 alleles per locus only with 14 or more loci, but it was possible with four or more loci when matings were known. At 16 loci, more than twofold more alleles per locus were required for 95% assignment success when matings were unknown than when they were known.

Actual parental and simulated offspring data

Simulation runs using actual data from the 1997 returns (Fig. 1) indicated that 96.7% (standard deviation (SD) = 0.5, $n = 8$) of the F2 offspring could be uniquely assigned to the correct set of four grandparents with known matings. Grandparentage assignment with unknown matings was less successful than with known matings. Average grandparentage assignment was 23.8% (SD = 7.0, $n = 8$) with unknown matings.

Table 2. Minimum number of alleles per locus required for >95% correct parentage assignment with unknown matings as a function of number of loci and number of parents.

Number of loci	Number of parents					
	10	50	110	210	310	410
4	10	16	16	18	>20	>20
6	6	8	8	10	10	12
8	<4	6	6	8	8	8
10	<4	6	6	6	6	6
12	<4	<4	<4	6	6	6
14	<4	<4	<4	<4	<4	<4
16	<4	<4	<4	<4	<4	<4

Note: Data are from simulated parent and simulated offspring computer runs with a normal parental allele distribution at each locus.

Discussion

The combination of parentage and grandparentage assignment in the Connecticut River Atlantic salmon restoration program provides a permanent, non-lethal, non-destructive, batch mark for families of small fish. The stocking of Atlantic salmon fry with known parentage can be used to identify characteristics of the fish (wild vs. hatchery, stocking date, stocking density, stocking size, and family differences in growth, survival, migration timing, and disease exposure) and of specific stocking tributaries (latitude, altitude, land use, aquatic community, temperature profiles, barriers to migration, etc.) that successfully produce smolts and adult returns. Smolts leaving the river and adults returning to the river can be trapped and assayed (using a non-lethal fin clip) for (grand)parentage assignment and therefore for any of the characteristics above.

In restoration efforts like the Connecticut River Atlantic salmon program where production of stocked fish depends on adult returns, adults are captured at hydroelectric facilities; this prevents homing to streams of origin. Assessing the relative contribution of various tributaries and their characteristics to the production of smolts and returns requires a mark at stocking, such as parentage assignment, or a natural tributary-specific mark. Thermal otolith marks (Volk et al. 1994) and coded wire tags (Blankenship 1990) have been used to batch-mark groups of fish, but identification requires lethal sampling. PIT tags have also been used extensively with Pacific salmon (Prentice et al. 1990), but Atlantic salmon are too small at stocking to support this physical tag. Kennedy et al. (1997, 2000) have developed a tributary-specific natural isotope marking technique that is effective for identifying small-scale movement patterns among tributaries but is less effective for evaluating basin-wide smolt and adult production.

Parentage assignment will be most effective for relatively small populations with localized spawning aggregations (e.g., ponds and anadromous fish in specific tributaries). Localized spawning allows collection of a large proportion of spawning adults. Because the proportion of identifiable individuals is lower with decreases in the proportion of genotyped parents, more "marked" individuals are likely to be recovered with a higher proportion of genotyped adults. Other population size limits to effective parentage assignment include increased logistical difficulties and increased costs associated with a larger number of fish. In the Connecticut River, we are currently genotyping 200–500 first-generation parents. Based on our simulations, parentage assignment should be possible with at least 10 000 first-generation parents with known matings (14 loci), but logistics and costs must be considered carefully before implementing a program with large numbers of fish. By combining parentage and grandparentage assignment, approximately 7 million Atlantic salmon fry (75% of total stocking) with known (grand)parentage will be stocked into the Connecticut River basin in 2002.

With all parentage assignment approaches, null alleles (O'Reilly et al. 1998) and mutations (Queller et al. 1993) can lead to incorrect assignment. Previous testing to identify deviations from H–W equilibrium (i.e., heterozygote deficiency) in Atlantic salmon populations using many of the loci reported herein (King et al. 2001; Spidle et al. 2001) has failed to detect null alleles. Mutation rates in microsatellite DNA have been reported to be 10^{-3} to 10^{-5} (Zhivotovsky and Feldman 1995) per generation. These findings suggest that null alleles and mutations are either absent or rare and should have minimal effects on assignment success. Similarly, O'Reilly et al. (1998) estimated a mutation rate of $\sim 3 \cdot 10^{-4}$ in a set of full-sib Atlantic salmon crosses and this had no effect on their ability to match offspring to true parents. Based on our error analysis, an error rate of 0.0003 would reduce assignment success to 0.990 compared with 0.998 with no error.

Sources of genotyping error include human handling error from initial sample acquisition, through DNA extraction, PCR amplification, electrophoresis, and fragment analysis. Compared with gel-based or parallel electrophoresis, a capillary-based, fully automated sequencer can reduce error by providing independence among samples, reducing gel anomalies and sample loading errors. Further, multiplexed PCR (coamplifying loci) minimizes genotyping error by reducing repeated handling of sample DNA. Implementing occasional checks (e.g., compatibility analysis, O'Reilly et al. 1998) has indicated that we have effectively minimized chances for human error in handling and genotyping the fish. Genotyping a subsample of the progeny from each paired mating will reveal process errors described above and germ line mutations. However, it should be noted that parentage assignment itself could also serve as a check for many such human errors. This error trapping should be an integral part of any large-scale parentage analysis operation.

Results from the simulations indicate the minimum numbers of loci and numbers of alleles per locus required for effective parentage and grandparentage assignment. These are minimum estimates because the allele values were generated from a smooth normal distribution and allele values commonly are not evenly distributed. Loci with uneven distributions of allele values (e.g., Ssa85, SSLEEN82) provide less discriminatory power for parentage assignment than loci with more evenly distributed allele values (e.g., Ssa171, Ssa202). Single alleles in loci such as SSLEEN82 and SsaD30 had frequencies greater than 0.5. These loci add little to our ability to identify parents and will ultimately be replaced by loci with more-evenly distributed alleles. Several additional hypervariable tri- and tetra-nucleotide loci have been developed recently for Atlantic salmon (Cairney et al. 2000; T.L. King, unpublished data). In addition to a large number of evenly distributed alleles, these higher-order microsatellite markers tend to result in fewer scoring errors owing to cleaner amplification and increased spacing between allelic categories. Application of these markers to a study such as this would enhance assignment success in parentage and grandparentage analysis. We suggest using our simulation results and those of Bernatchez and Duchesne (2000) as a rough guideline for determining the likelihood of a successful parentage assignment and the testing of actual data, if available, before implementing an assignment program.

Our simulation results showed that differences in parentage assignment success with known and unknown matings depended on genetic information content. With low genetic information content (number of loci, alleles per locus), there were substantial differences between assignment success with known and unknown matings. Once there were more than 10 loci and five alleles per locus however, the difference in assignment success between known and unknown matings shrank to zero. In the hatchery, it may not be worth the extra effort of tracking known matings if enough sufficiently polymorphic loci are available. In our Connecticut River Atlantic salmon example, it is necessary to track all first-generation matings despite access to hypervariable loci because we need to perform grandparentage analysis of the F2 offspring. In the wild, where matings are usually unknown, it will be important to consider the proportion of mating individuals that was genotyped to further assess the applicability of parentage analysis. In cases where a large proportion of mating individuals cannot be genotyped, parentage analysis may be difficult without a large number of hypervariable genotypes combined with alternative analyses that account for incomplete sampling (Neff et al. 2000). Preliminary, unpublished data (B.H. Letcher) indicate that successful parentage assignment of offspring from 100 genotyped candidate parents (14 loci, 12 alleles per locus) is possible when the genotyped parents are mixed with up to at least 5000 untyped parents with randomly generated genotypes. Further analysis should explore the effects of variation in the number of ungenotyped parents on parentage analysis of candidate parents with known genotypes.

Acknowledgements

We thank Mike Kinnison, Andrew Hendry, Dave Perkins, Adrian Spidle, Stephanie Carlson, Gregg Horton, Keith Nislow, Elizabeth Marschall, and three anonymous reviewers for helpful comments on the manuscript. W.B. Schill and J.A. Sanchez Prado allowed use of unpublished markers. We also thank the numerous state and federal agency personnel who assisted in this program. Ken Gillette (USFWS, White River National Fish Hatchery), Steve Gephard (State of Connecticut, Department of Environmental Protection), and Mickey Novak (USFWS, Richard Cronin National Salmon Station) were particularly involved and helpful.

References

Achord, S., Matthews, G.M., Johnson, O.W., and Marsh, D.M. 1996. Use of passive integrated transponder (PIT) tags to monitor migration timing of Snake River chinook salmon smolts. N. Am. J. Fish. Manag. **16**: 302–313.

Allendorf, F.W., Bayles, D., Bottom, D.L., Currens, K.P., Frissell, C.A., Hankin, D., Lichatowich, J.A., Nehlsen, W., Trotter, P.C., and Williams, T.H. 1997. Prioritizing Pacific salmon stocks for conservation. Conserv. Biol. **11**: 140–152.

Angers, B., Bernatchez, L., Angers, L., and Desgroseilliers, L. 1995. Specific microsatellite loci for brook charr reveal strong population subdivision on a microgeographic scale. J. Fish Biol. **47**: 177–185.

Ballou, J.D., Gilpin, M., and Foose, J. 1995. Population management for survival and recovery: analytical methods and strategies in small population conservation. Columbia University Press, New York.

Bernatchez, L., and Duchesne, P. 2000. Individual-based genotype analysis in studies of parentage and population assignment: how many loci, how many alleles? Can. J. Fish. Aquat. Sci. **57**: 1–12.

Bernatchez, L., and Wilson, C.C. 1999. Diadromy and genetic diversity in Nearctic and Palearctic fishes: a reply. Mol. Ecol. **8**: 529–530.

Blanchfield, P.J., and Jones, M.W. 2001. Reproductive success in salmonids. Rev. Fish Biol. Fish. In press.

Blankenship, H.L. 1990. Effects of time and fish size on coded wire tag loss from chinook and coho slamon. Am. Fish. Soc. Symp. **7**: 237–243.

Cairney, M., Taggart, J.B., and Høyheim, B. 2000. Characterization of microsatellite and minisatellite loci in Atlantic salmon (*Salmo salar* L.) and cross-species amplification in other salmonids. Mol. Ecol. **9**: 2175–2178.

Chakraborty, R., Meagher, T.R., and Smouse, P.E. 1988. Parentage analysis with genetic markers in natural populations. I. The expected proportion of offspring with unambiguous paternity. Genetics, **118**: 527–536.

Estoup, A., Gharbi, K., SanCristobal, M., Chevalet, C., Haffray, P., and Guyomard, R. 1998. Parentage assignement using microsatellites in turbot (*Scophthalmus maximus*) and rainbow trout (*Oncorhynchus mykiss*) hatchery populations. Can. J. Fish. Aquat. Sci. **55**: 715–725.

Fleming, I.A., Hindar, K., Mjølnerød, I.B., Jonsson, B., Balstad, T., and Lamberg, A. 2000. Lifetime success and interactions of farm salmon invading a native population. Proc. Roy. Soc. Lond. B, Biol. Sci. **267**: 1517–1523.

Garcia de Leon, F., Canonne, M., Quillet, E., Bonhomme, F., and Chatain, B. 1998. The application of microsatellite markers to breeding programmes in the sea bass, *Dicentrarchus labrax*. Aquaculture, **159**: 303–316.

Herbinger, C.M., O'Reilly, P.T., Doyle, R.W., Wright, J.M., and O'Flynn, F. 1999. Early growth performance of Atlantic salmon full-sib families reared in single family tanks versus in mixed family tanks. Aquaculture, **173**: 105–116.

Kennedy, B.P., Folt, C., Blum, J.D., and Chamberlain, C.P. 1997. Natural isotope markers in salmon. Nature (London), **387**: 766–767.

Kennedy, B.P., Blum, J.D., Folt, C.L., and Nislow, K.H. 2000. Using natural strontium isotopic signatures as fish markers: methodology and application. Can. J. Fish. Aquat. Sci. **57**: 2280–2292.

Kennedy, G.J.A. 1988. Stock enhancement of Atlantic salmon (*Salmo salar* L.). *In* Atlantic salmon: planning for the future. *Edited by* D. Mills and D. Piggins. Timber Press, Portland, Oreg. pp. 345–372.

King, T.L., Kalinowski, S.T., Schill, W.B., Spidle, A.P., and Lubinski, B.A. 2001. Population structure of Atlantic salmon (*Salmo salar* L.): a range-wide perspective from microsatellite DNA variation. Mol. Ecol. **10**: 807–82

Labarca, C., and Paigen, K. 1980. A simple, rapid, and sensitive DNA assay procedure. Anal. Biochem. **102**: 344–352.

Marshall, T.C., Slate, J., Kruuk, L.E.B., and Pemberton, J.M. 1998. Statistical confidence for likelihood-based paternity inference in natural populations. Mol. Ecol. **7**: 639–655.

Martinez, J.L., Moran, P., Perez, J., De Gaudemar, B., Beall, E., and Garcia-Vazquez, E. 2000. Multiple paternity increases effective size of southern Atlantic salmon populations. Mol. Ecol. **9**: 292–298.

McDowall, R.M. 1999. Diadromy and genetic diversity in Nearctic and Palearctic fishes. Mol. Ecol. **8**: 527–528.

Moring, J.R., Marancik, J., and Griffiths, F. 1995. Changes in stocking strategies for Atlantic salmon restoration and rehabilitation in Maine, 1871–1993. Am. Fish. Soc. Symp. **15**: 38–46.

Neff, B.D., Repka, J., and Gross, M.R. 2000. Parentage analysis with incomplete sampling of candidate parents and offspring. Mol. Ecol. **9**: 515–528.

Norris, A.T., Bradley, D.G., and Cunningham, E.P. 2000. Parentage and relatedness determination in farmed Atlantic salmon (*Salmo salar*) using microsatellite markers. Aquaculture, **182**: 73–83.

O'Reilly, P.T., Hamilton, L.C., McConnell, S.K., and Wright, J.M. 1996. Rapid analysis of genetic variation in Atlantic salmon (*Salmo salar*) by PCR multiplexing of dinucleotide and tetranucleotide microsatellites. Can. J. Fish. Aquat. Sci. **53**: 2292–2298.

O'Reilly, P.T., Herbinger, C., and Wright, J.M. 1998. Analysis of parentage determination in Atlantic salmon (*Salmo salar*) using microsatellites. Anim. Genet. **29**: 363–370.

Prentice, E.F., Flagg, T.A., and McCutcheon, C.S. 1990. Feasibility of using implantable passive integrated transponder (PIT) tags in salmonids. Am. Fish. Soc. Symp. **7**: 317–322.

Queller, D.C., Strassmann, J.E., and Hughes, C.R. 1993. Microsatellites and kinship. Trends Ecol. Evol. **8**: 285–288.

Raymond, M., and Rousset, R. 1995. GENEPOP (version 1.2): population genetics software for exact tests and ecumenicism. J. Hered. **86**: 248–249.

SanCristobal, M., and Chevalet, C. 1997. Error tolerant parent identification from a finite set of individuals. Genet. Res. **70**: 53–62.

Schramm, H.L., Jr., and Piper, R.G. 1995. Uses and effects of cultured fishes in aquatic ecosystems. *In* American Fisheries Society Symposium 15. AMS, Bethesda, Md.

Schroder, S.L., Knudsen, C.M., and Volk, E.C. 1995. Marking salmon fry with strontium chloride solutions. Can. J. Fish. Aquat. Sci. **52**: 1141–1149.

Slettan, A., Olsaker, I., and Lie, O. 1995. Atlantic salmon, *Salmo salar*, microsatellites at the SSOSL25, SSOSL85 and SSOSL311, SSOSL417 loci. Anim. Genet. **26**: 281–282.

Slettan, A., Olsaker, I., and Lie, O. 1996. Polymorphic Atlantic salmon, *Salmo salar* L., microsatellites at the SSOSL438, SSOSL439 and SSOSL444 loci. Anim. Genet. **27**: 57–64.

Spidle, A.P., Schill, W.B., Lubinski, B.A., and King, T.L. 2001. Fine-scale population structure in Atlantic salmon from Maine's Penobscot River drainage. Conserv. Genet. **2**: 11–24.

Volckaert, F.A.M., and Hellemans, B. 1999. Survival, growth and selection in a communally reared multifactorial cross of African catfish (*Clarias gariepinus*). Aquaculture, **171**: 49–64.

Volk, E.C., Schroder, S.L., and Grimm, J.J. 1994. Use of a bar code symbology to produce multiple thermally induced otolith marks. Trans. Am. Fish. Soc. **123**: 811–816.

Waples, R. 1991. Genetic interactions between hatchery and wild salmonids: lessons from the pacific northwest. Can. J. Fish. Aquat. Sci. **48**(Suppl. 1): 124–133.

Wolfus, G.M., Garcia, D.K., and Alcivar-Warren, A. 1997. Application of the microsatellite technique for analyzing genetic diversity in shrimp breeding programs. Aquaculture, **152**: 35–47.

Zhivotovsky, L.A., and Feldman, M.W. 1995. Microsatellite variability and genetic distances. Proc. Natl. Acad. Sci. U.S.A. **92**: 11 549 – 11 552.

Appendix

Following the nomenclature of SanCristobal and Chevalet (1997), we extend parentage assignment through two generations so that we assign F2 offspring to their P1 grandparents. In the first generation, the probability of an offspring with genotype $[A_iA_j]$ deriving from two putative parents with genotypes (A_aA_b) (A_cA_d) is written as

$$\Pr([A_iA_j] | \ (A_aA_b),(A_cA_d)) = T(i/ab)\cdot T(j/cd) + T(j/ab)\cdot T(i/cd)$$

where $T(i/ab) = \Pr((A_i)|(A_aA_b)) = \frac{1}{2}(a = i) + \frac{1}{2}(b = i)$. $(a = i)$ is a Boolean operator that gives a value of 1 if the allele value of a equals the allele value of i. If they are unequal, it gives a value of zero. If the offspring is a homozygote $(a = b)$, $\Pr([A_iA_j] | \ (A_aA_b),(A_cA_d))$ is divided by 2 to give the locus-specific likelihood, otherwise it is divided by 1. The global likelihood for the offspring conditional on the parental pair is the product of all single locus likelihoods.

Extending the analysis to examine a set of four grandparents (A_kA_l) (A_mA_n) (A_qA_r) (A_sA_t) gives

$$\begin{aligned}\Pr([A_iA_j] | \ (A_kA_l)\ (A_mA_n)\ (A_qA_r)\ (A_sA_t)) = {} & [T(i/kl)\cdot T(j/mn) + T(j/kl)\cdot T(i/mn)] \\ & + [T(i/kl)\cdot T(j/qr) + T(j/kl)\cdot T(i/qr)] \\ & + [T(i/kl)\cdot T(j/st) + T(j/kl)\cdot T(i/st)] \\ & + [T(i/mn)\cdot T(j/qr) + T(j/mn)\cdot T(i/qr)] \\ & + [T(i/mn)\cdot T(j/st) + T(j/mn)\cdot T(i/st)] \\ & + [T(i/qr)\cdot T(j/st) + T(j/qr)\cdot T(i/st)]\end{aligned}$$

The global likelihood is then calculated as above. A global likelihood must be calculated for all sets of four potential grandparents. The set with the maximum likelihood is scored as the most likely set and, when P1 parent sets are known, can be compared to the actual set.

Recovery of zooplankton assemblages in mountain lakes from the effects of introduced sport fish

David B. Donald, Rolf D. Vinebrooke, R. Stewart Anderson, Jim Syrgiannis, and Mark D. Graham

Abstract: Impacts of introduced fish on zooplankton assemblages of lakes may persist for decades following fish removal. We tested this hypothesis by comparing zooplankton assemblages from four categories of lakes located in western Canadian mountain parks including lakes without and with fish that differed in their fish community complexity and fish-stocking history. Zooplankton species richness was greatest in lakes with a complex community of fish and least in pristine fishless lakes. Canonical correspondence analysis showed that taxonomic shifts in zooplankton assemblages could be attributed to differences in fish-stocking history between the study lakes. In fishless lakes, larger copepods (*Eucyclops agilis*, *Diaptomus leptopus*), cladocerans (*Diaphanosoma*, large *Daphnia*), and chaoborids were abundant, whereas in the presence of fish, small crustaceans were more common and chaoborids were relatively rare. Once introduced trout were absent from lakes, recovery trajectories for zooplankton showed a general taxonomic shift towards assemblages characteristic of fishless lakes that had never been fish stocked. Based on separation between previously stocked fishless lakes and naturally fishless mountain lakes in ordination space (chi-squared distance), taxonomic recovery by zooplankton assemblages from the influence of introduced salmonids may require an average of 19 years.

Résumé : L'impact de l'introduction de poissons sur les communautés de zooplancton dans les lacs peuvent se faire sentir des dizaines d'années après le retrait des poissons. Une comparaison de communautés zooplanctoniques de quatre catégories de lacs dans les parcs montagneux de l'Ouest canadien, dont des lacs avec et sans poissons qui différaient dans la complexité de leur communauté de poissons et leur régime d'ensemencement, a permis d'éprouver cette hypothèse. La richesse spécifique du zooplancton est plus importante dans les lacs à communauté de poissons complexe et moindre dans les lacs non-pollués sans poissons. Une analyse canonique des correspondances indique que les changements taxonomiques dans les communautés zooplanctoniques peuvent être attribués à des différences dans le régime des ensemencements dans les lacs étudiés. Dans les grands lacs sans poissons, les copépodes (*Eucyclops agilis*, *Diaptomus leptopus*), les cladocères (*Diaphanosoma*, grandes espèces de *Daphnia*) et les chaoboridés sont abondants, alors qu'en présence de poissons, les petits crustacés sont plus nombreux et les chaoboridés sont relativement rares. Lorsque la truite introduite est retirée d'un lac, la trajectoire de récupération du zooplancton montre un glissement taxonomique général vers les communautés caractéristiques de lacs sans poissons qui n'ont jamais été ensemencés. D'après la séparation des lacs de montagne ensemencés et des lacs naturellement sans poissons dans l'espace réduit (distance «chi-squared»), le rétablissement taxonomique des communautés de zooplancton des effets d'une introduction de salmonidés peut requérir en moyenne une période de 19 années.

[Traduit par la Rédaction]

Introduction

Fish affect zooplankton assemblages in lakes by size-selective predation on chaoborids and large species of copepods and cladocerans (von Ende 1979; Mittelbach et al. 1995). As a result, fish predation often increases the diversity and abundance of small crustaceans. For example, zooplanktivory by introduced fish, such as rainbow trout (*Oncorhynchus mykiss*), brook trout (*Salvelinus fontinalis*), and alewife (*Alosa pseudoharengus*) typically causes a size shift towards smaller invertebrate species in lakes (Brooks and Dodson 1965; Anderson 1972).

Zooplankton assemblages can show strong resilience to manipulations of complex fish communities in temperate lakes. For example, Mittelbach et al. (1995) concluded that the capacity of an altered zooplankton assemblage to return to the previous state was not restricted. Specifically, zooplankton species composition and mean body size were changed dramatically by increased planktivory by fish over a period of 7 years following the removal of the dominant predator, largemouth bass (*Micropterus salmoides*). However, the zooplankton community returned to its previous state within a few years following the reintroduction of bass. Similar compensatory dynamics within complex fish and zooplankton assemblages have been reported following the

Received December 22, 2000. Accepted June 8, 2001. Published on the NRC Research Press Web site at http://cjfas.nrc.ca on August 25, 2001.
J16147

D.B. Donald[1] **and J. Syrgiannis.** Environment Canada, Room 300 Park Plaza, 2365 Albert Street, Regina, SK S4P 4K1, Canada.
R.D. Vinebrooke and M.D. Graham. Department of Biology, University of Regina, Regina, SK S4S 0A2, Canada.
R.S. Anderson. P.O. Box 127, New Sarepta, AB T0B 3M0, Canada.

[1] Corresponding author (e-mail: David.Donald@ec.gc.ca).

DOI: 10.1139/cjfas-58-9-1822

termination of trout stocking in a montane lake (Elser et al. 1995).

In contrast, zooplankton in pristine fishless alpine lakes can show low resilience following the extinction of introduced sport fish (Parker et al. 1996; McNaught et al. 1999). In this case, the omnivorous copepod *Hesperodiaptomus arcticus* had not recolonized an alpine lake in western Canada 26 years after trout were last stocked and 8 years after fish were last caught. Similarly, introduced trout have had profound adverse effects on zooplankton species diversity and composition in California alpine lakes (Bradford et al. 1998). Topographic barriers may impede dispersal and recovery by zooplankton species in mountain lakes compared with other lake regions where the zooplankton communities appear to be species-saturated (see Shurin et al. 2000). Therefore, we hypothesized that if introduced sport fish persist in lakes for decades before becoming extinct, their impact on zooplankton assemblages may in turn persist for decades. We tested this hypothesis by examining relationships between zooplankton and fish assemblages of fishless and fish-inhabited mountain lakes in their pristine condition and in lakes where sport fish had been stocked and subsequently become extinct.

Materials and methods

Study area

Most lakes in the national parks of the Rocky Mountains of Alberta and British Columbia, Canada, were devoid of fish at the beginning of the twentieth century (Donald 1987). Many of these lakes were subsequently stocked for several decades with salmonids. Exotic sport fish established self-recruiting populations in some lakes, while other populations were maintained by annual introductions of fingerlings. Beginning in the 1970s, sport-fish stocking was stopped for lakes that were either too remote, lacked suitable habitat conditions (e.g., winter anoxia), or supported populations of sport and other fish species maintained by natural recruitment. In 1988, sport-fish stocking was discontinued in Canadian national parks because it was deemed to be ecologically inappropriate.

Mid-summer zooplankton samples were collected over 29 years from 25 lakes situated along the Front Range of the Rocky Mountains in Jasper, Banff, and Kootenay national parks of Alberta and British Columbia, Canada. The lakes were all within the montane (1030–1500 m above sea level (ASL)) and lower subalpine (1501–1692 m ASL) ecozones (Table 1). The lakes included in this study were 0.4–32.3 ha in area with maximum depth ranging from 3 to 25 m, total dissolved solids from 39 to 278 $\mathrm{mg \cdot L^{-1}}$, and dissolved organic carbon from 1.2 to 18.5 $\mathrm{mg \cdot L^{-1}}$. Mid-summer water temperature was measured at each lake once each year from 1 to 20 August and was calculated as the mean temperature for the zone from 2 to 10 m deep or from 2 m to the maximum depth for lakes less than 10 m deep. Water samples for chemical analyses were collected near the centre of each lake at a depth of 0.4 or 5.0 m and were analyzed by methods given in Anonymous (1994).

Sampling of fish and zooplankton

Fish and zooplankton assemblages were determined for each lake in four categories: (*i*) five pristine "reference" lakes that had never been stocked or supported any fish; (*ii*) seven lakes with a relatively complex fish community of self-maintained indigenous and introduced fish (Catostomidae, Cyprinidae, and Salmonidae); (*iii*) eight lakes with only salmonid populations that were maintained by frequent stocking of hatchery-reared fish because these lakes did not have suitable spawning habitat for salmonids; and (*iv*) 13 fishless lakes that had not been stocked for 8 to 30 years and had no or few fish, including the previous eight lakes after they had not been stocked for 8 to 23 years (Table 1). In their pristine state, these 13 lakes would have been fishless (Donald 1987). Once stocking of salmonids was discontinued, 8 or more years of natural and fishing mortality reduced salmonid abundance substantially. For example, gill nets set in Kootenay Pond, Little Honeymoon, Kingfisher, Hibernia, Golden, and Marjorie lakes, 6 to 13 years after the last fish were stocked, did not yield fish of any species. However, a few old trout were caught in Upper Colfair and Dragon lakes 8 years after the last fish were stocked. Moreover, surveys of dozens of montane and subalpine lakes have shown that the maximum longevity for rainbow trout was 9 years, for cutthroat trout, 11 years, and for brook trout, 13 years (Donald et al. 1980; Donald and Alger 1986). Five of the 13 lakes (Colfair, Cutt, Dragon, Golden, and Little Honeymoon) were stocked infrequently, and their zooplankton assemblages may not have been measurably affected by the presence of fish. Zooplankton populations in these five lakes were sampled from 8 to 30 years after fish were last stocked. Only five pristine reference lakes were sampled because relatively deep, low-elevation fishless lakes (>3 m maximum depth) were rare in our study area. However, these and other lakes included in our study were representative of the small, low-elevation lakes in the region.

The status of the fish community was determined using gill nets for most of the study lakes in the 1930s (D.S. Rawson (deceased), Department of Biology, University of Saskatchewan, Saskatoon, Sask., unpublished data) and for all lakes with fish in the 1970s and early 1980s (Donald and Anderson 1982; Donald 1987). For the 13 stocked lakes, absence of natural recruitment for salmonids was determined in the 1970s and 1980s from the size and age of fish in the catches plus the presence in the catches of only hatchery-reared fish that were marked before being released into the lakes. Additional gill-net catches were made in 10 of the lakes in the 1990s to confirm fish population status. The national park stocking records were used to identify the five pristine reference lakes (no records of stocking or fish presence) and to determine the number of years stocked salmonids were present in other lakes. These records were maintained for each lake from the early 1900s to 1988 when salmonids were last stocked in the national parks. We assumed that each time fish were stocked in a lake, a significant population of trout would persist for 6 years.

Zooplankton were collected during mid-summer using Wisconsin-style plankton nets in the 1970s and early 1980s (aperture size 65 μm^2, diameter 25 cm, length 80 cm) and in the 1990s (aperture size 63 μm^2, diameter 30 cm, length 100 cm). Vertical hauls were made at a rate of 0.5 $\mathrm{m \cdot s^{-1}}$ from the deepest part of the lake. Crustacean and chaoborid plankton were identified and counted, and densities per litre were determined according to the procedures outlined in Anderson (1974). The total number of zooplankton species (i.e., species richness) was considered as an index, rather than as an absolute measure, of zooplankton diversity in each lake because single-sample enumerations can seriously underestimate total species in lakes by up to 50% (Arnott et al. 1998).

Statistical analyses

A two-factor (fish presence × fish-stocking history) experimental design was used to test for the effects of fish and salmonid stocking on zooplankton species richness in the study lakes (Table 1). Four treatment combinations were made possible using two levels of fish-stocking history (salmonid stocked vs. pristine) and fish presence (fish present vs. fish absent). Specifically, five lakes (K13, K20, J392, J436, and J439) comprised the non-stocked, pristine fishless lake category. Seven lakes (Annette, Beauvert, Dog, Dorothy, Iris, Outram, and Vista) represented lakes with a relatively complex fish community. Eight lakes that were stocked frequently and sampled during active stocking in the 1970s consti-

Table 1. Morphometry, elevation, water chemistry, temperature, and fish populations of montane and lower subalpine lakes.

			Morphometry			Chemistry		
Lake	Latitude (north)	Longitude (west)	Area, ha	Maximum depth, m	Elevation, m	TDS, mg·L^{-1}	DOC, mg·L^{-1}	Mid-summer temperature, °C
No fish present and never stocked								
K13	50°53′	116°05′	2.5	6.0	1295	219	10.5	15.5
K20	50°50′	116°02′	0.6	3.8	1218	278	8.3	no data
J392	52°51′	118°03′	0.6	8.0	1117	212	14.9	10.0
J436	52°54′	118°19′	7.5	3.0	1455	135	18.5	no data
J439	52°55′	118°22′	7.1	3.0	1615	52	9.3	15.9
Complex community of Catostomidae, Cyprinidae, and Salmonidae								
Annette	52°54′	118°02″	28.6	23.0	1024	153	1.9	17.1
Beauvert	52°53′	118°03′	32.3	25.0	1030	135	1.2	12.4
Dog	50°48′	115°57′	15.1	4.7	1183	183	3.7	19.7
Dorothy	52°53′	118°14′	25.1	14.6	1336	145	6.8	13.1
Iris	52°52′	118°13′	5.1	13.2	1302	134	6.2	14.5
Outram	51°52′	116°48′	9.4	11.5	1460		3.3	17.8
Vista	51°14′	116°01′	6.6	6.5	1567	130	1.8	12.2
Salmonid populations maintained by introducing hatchery-reared fish								
Colfair (Upper)	52°44′	117°52′	2.9	7.5	1555	53	8.0	15.7
Copper	51°15′	155°55′	1.5	10.4	1434	86	10.5	17.1
Cutt	52°53′	118°20′	15.5	7.5	1455	62	5.9	14.4
Dragon	52°28′	117°40′	15.6	12.0	1341	100	3.0	18.2
Golden	52°53′	118°20′	13.4	10.3	1485	39	6.0	14.9
Hibernia	52°52′	118°08′	9.6	8.5	1198	158	9.9	16.9
Honeymoon (Little)	52°34′	117°41′	1.0	4.0	1310	146	9.0	19.7
Kingfisher	51°24′	116°10′	2.0	7.2	1539	113	10.7	16.0
Kootenay Pond	50°53′	116°02′	2.6	6.0	1213	234	5.4	16.1
Lost	51°28′	116°16′	0.4	5.5	1692	163	4.9	12.9
Marjorie	52°52′	118°08′	7.3	8.0	1143	179	9.0	16.7
Pilot	51°14′	115°49′	2.8	7.5	1408	202	8.2	15.6
Virl	52°52′	118°14′	3.5	6.0	1305	123	9.5	17.9

Note: TDS, total dissolved solids; DOC, dissolved organic carbon; —, stocking infrequent.

[a] LS, longnose sucker (*Catostomus catostomus*); LC, lake chub (*Couesius plumbeus*); BT, brook trout (*Salvelinus fontinalis*); WS, white sucker (*Catostomus commersoni*); MW, mountain whitefish (*Prosopium williamsoni*); RT, rainbow trout (*Oncorhynchus mykiss*); LT, lake trout (*Salvelinus namycush*); CT, cuttroat trout (*O. clarki*); BU, bull trout (*Salvelinus confluentus*).

[b] Assumes that fish were common for 6 years after each introduction of hatchery-reared fish.

tuted the salmonid-inhabited lake category, and these same eight lakes plus five other stocked lakes that were visited in the mid-1990s after salmonid stocking had been stopped were used to construct the manipulated, fishless lake category. However, the total number of samples was 14 because Kootenay Pond was sampled twice, 9 and 24 years after the last introduction of fingerlings. A 2-way ANOVA with multiple comparison (Bonferroni–Dunn) testing was performed to test for the significance of the response by zooplankton to the presence of fish and fish-stocking events. Data analysis was performed using StatView® version 5 (SAS Institute, Cary, N.C).

The zooplankton and fish taxonomic data were analyzed using canonical correspondence analysis (CCA). Species densities were $\log_{10}(x+1)$ transformed to reduce the influence of abundant taxa that otherwise dominate ordination analyses. Fish status (1, natural fishless reference; 2, complex fish community; 3, stocked with salmonids; and 4, fishless after salmonid stocking ceased) of each lake at the time of sampling was used as an environmental variable consisting of four categorical levels for the CCA. Environmental data, including fish status, lake area and maximum depth, total dissolved solids (TDS), dissolved organic carbon (DOC), and mid-summer temperature, were screened for insignificant variables by testing the significance of partial CCAs that were each performed using only one of the environmental variables (Vinebrooke and Graham 1997). Following the elimination of insignificant variables, a final CCA was performed and the significance of the ordination was determined using Monte Carlo permutation testing. Ordinations were performed using CANOCO version 3.12 (ter Braak 1991).

Simple regression analysis was performed to determine if taxonomic recovery by zooplankton assemblages from the effects of introduced sport fish could be predicted based on the number of years since a lake had last been stocked. The metric used to measure taxonomic recovery was chi-squared distance between CCA biplot coordinates for those eight salmonid-stocked lakes that were resampled 8–23 years after they had returned to a fishless state following the termination of salmonid stocking.

Results

Fish status of survey lakes

Salmonids were introduced into 13 lakes that were originally fishless by stocking one or more species including rainbow trout and brook trout (Table 1). Salmonids were present in these lakes continuously over periods of 6–51 years. The 13 lakes were sampled for zooplankton following the termination of fish stocking and the time-related reduction of their

Fish populations					
Indigenous or introduced[a]	Number of years fish abundant[b]	Mean stocking density, number·ha^{-1}	Maximum stocking density, number·ha^{-1}	Year last stocked	Year(s) plankton collected
					1996
					1996
					1997
					1995
					1996
LS,LC,BT					1994
LS,WS,MW,RT,BT					1994
LS,BT,RT					1981
LS,LC,RT					1978
LS,LC,RT					1994
LS,LC,LT,BT					1995
LS,BT,CT,BU					1994
RT	6	—	170	1971	1979
RT	35	930	2 330	1988	1973/96
RT	11	—	300	1986	1995
BT	18	—	170	1971	1979
RT	19	—	370	1986	1998
RT	51	740	1 560	1986	1970/94
BT	9	—	1 000	1966	1996
RT	45	600	5 000	1988	1973/96
BT,RT	24	560	1 115	1972	1972/81/96
BT	15	250	25 000	1972	1970/95
RT	51	1230	2 465	1986	1970/94
BT,RT	35	1240	1 785	1985	1970/96
RT	15	510	1 430	1986	1970/94

introduced fish populations (Table 1). The maximum number of fish introduced into these lakes for 1 year ranged from 170 to 25 000 fish·ha^{-1}. During the 1970s, zooplankton samples were collected from eight of these lakes when they were stocked annually at densities of 250–1240 fish·ha^{-1}.

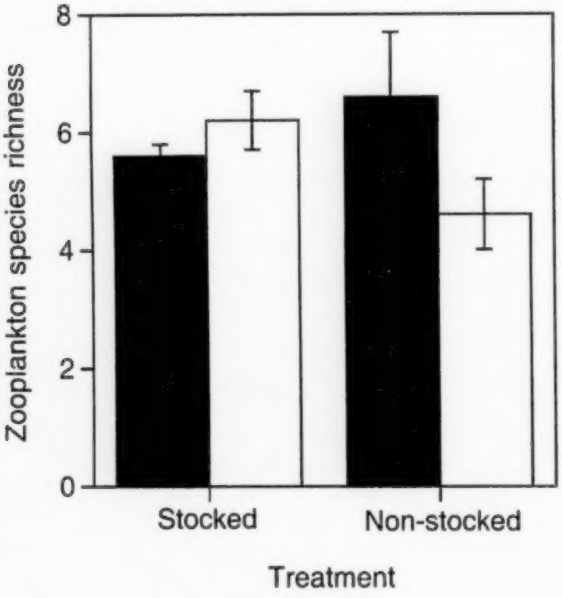
Zooplankton status

Zooplankton species richness was suppressed by salmonids in stocked lakes and, conversely, enhanced by the presence of indigenous fish species in other lakes ($F_{1,30}$ = 4.31, P = 0.04; Fig. 1). However, the mean number of copepod species was similar for lakes with and without fish (Table 2). Cladoceran species diversity was greater in lakes with a complex fish assemblage (mean 4.3 species, standard deviation (SD) = ±2.56, N = 7) compared with all fishless lakes (mean 2.4 species, SD = ±1.20, N = 18, Student's t test = 2.57, $P < 0.05$) and was intermediate for lakes with stocked salmonid populations (mean 3.3 species, N = 8). Cladoceran species diversity was similar for the two groups of lakes that did not have fish (range for mean 2.2 to 2.5 species). Chaoborid species diversity was less in lakes with a complex fish assemblage (mean 0.29 species/lake) compared with all fishless lakes (mean 1.4 species/lake, t = 3.34, $p < 0.05$). Common species were not restricted to lakes either with or without fish, although *Daphnia rosea* was more common in lakes with fish compared with all fishless lakes (10 of 15 and 1 of 19 samples, respectively, z test of proportions = 3.81, $p < 0.05$). This pattern was similar for *Bosmina longirostris* (8 of 15 samples from lakes with fish and 1 of 19 samples from lakes without fish, z = 3.16) and for *Ceriodaphnia* (7 of 15 and 1 of 19, respectively, z = 2.84).

The five "reference" lakes, which had never been stocked and did not support indigenous fish species, had high abundances of *Diaptomus leptopus*, *Chaoborus americanus*, and large *Daphnia* (Table 3). The dominant cladocerns were large *D. pulex* or *pulicaria* or *Diaphanosoma leuchtenbergianum*. The mean number of copepod and cladoceran species was 2.2 for both of these crustacean groups.

Zooplankton community composition was more diverse and variable across the seven lakes that contained complex fish assemblages (Table 2). *Diaptomus leptopus* was present in three of the seven lakes with a complex fish assemblage of indigenous and introduced species. However, in contrast to pristine fishless lakes, the dominant copepods were

Fig. 1. Effects of sport-fish stocking and fish status (fish present, solid bars; fish absent, open bars) on zooplankton species richness in the surveyed mountain lakes. Error bars represent ±1 standard error.



cyclopoids (*Diacyclops thomasi*, *Orthocyclops modestus*, or *Macrocyclops albidus*) (Table 2). The dominant cladoceran was large *Daphnia*, *Ceriodaphnia*, *Bosmina longirostris*, or *Daphnia rosea*. Chaoborids (*C. flavicans*) were found in only two of the seven lakes. The mean number of copepod and cladoceran species in these lakes was 2.7 and 4.3, respectively.

Stocked lakes had an average of 5.6 crustacean zooplankton taxa. *Diaptomus leptopus* was present in five of the eight lakes and was the dominant copepod in three lakes. In the remaining lakes, one of four other copepod species was dominant (Table 2). The dominant cladoceran taxon was a large *Daphnia* species (25%), *Ceriodaphnia*, *Bosmina longirostris*, or *Daphnia rosea*.

Eight to 12 years after salmonid stocking ceased, *D. leptopus* was present in 9 of 11 fishless lakes and was the dominant copepod in five lakes or 46% (Table 2). *Acanthodiaptomus denticornis* or *Acanthocyclops vernalis* was dominant in the other six lakes. The dominant cladoceran taxa in all of these lakes were either large *Daphnia* or *Diaphanosoma*. Chaoborids were present in all 11 lakes.

From 23 to 30 years after stocking ceased, *D. leptopus* was present in all three lakes and was the dominant copepod in two of the three lakes (Honeymoon and Kootenay Pond). *Acanthodiaptomus denticornis* was the dominant copepod in the other lake (Lost Lake, Table 2). Only large *Daphnia* or *Diaphanosoma* were the dominant cladoceran in these lakes, and all supported one or more species of *Chaoborus* (maximum four species).

Ordination analysis

A CCA of invertebrate and fish data showed a significant ($P = 0.01$) relationship between zooplankton assemblages and the fish-stocking histories of the study lakes (Fig. 2). The first CCA axis captured 29% of the variance contained within the species data and contrasted large-bodied zooplankton (*Chaoborus* and large daphnids) in fishless lakes from small-bodied zooplankton (*Daphnia rosea* and *Diaptomus tyrelli*) in fish-inhabited lakes. The second CCA axis accounted for 5% of the variance in the species data and separated lakes that had been stocked with fish from those that contained few or no introduced fish. Fish-stocking history was retained as the only environmental variable following screening of the data for insignificant variables.

Zooplankton assemblages changed substantially in those mountain lakes where salmonid populations were no longer maintained by annual stocking (Fig. 2). Fish-stocked lakes that had returned to a fishless state showed a common shift in ordination space towards the right (Fig. 2). Several lakes (Kingfisher, Hibernia, Kootenay, Lost, and Virl) also shifted upwards in ordination space towards the naturally fishless lakes in the upper right quadrant of the plot (Fig. 2). Chi-squared distance between CCA coordinates for salmonid-stocked lakes resampled after they had returned to a fishless state (Fig. 2) was significantly related to the number of years since the last recorded stocking event ($\log_{10}$(chi-squared distance) = 0.033 years, $r^2 = 0.76$, $F_{1,8} = 25.49$, $p = 0.001$). As a result, total chi-squared distance between the centroids representing salmonid-stocked lakes, stocked lakes that had returned to a fishless state, and pristine fishless lakes (Fig. 2*a*) equates to 18.3 years.

Discussion

Introduction of salmonid sport fish probably altered zooplankton assemblages in montane and lower subalpine lakes that were originally fishless. These fish-induced changes were evident from a comparison of zooplankton assemblages of lakes with and without fish. The plankton species found in stocked lakes were often the same species present in lakes with indigenous fish communities. However, stocked lakes contained significantly fewer zooplankton species than lakes that contained indigenous fish species. A possible explanation for greater zooplankton diversity in the presence of a native fish community is that predation on zooplankton by a variety of fish species of different sizes and feeding strategies, rather than a single species, reduced niche breadth and dominance of individual plankton species, permitting rarer species to become established in the plankton community.

Differences in zooplankton assemblages of lakes with and without fish were likely related to size-selective predation by fish on chaoborid and large crustacean plankton (Brooks and Dodson 1965; Northcote 1988; Mittelbach et al. 1995). Certainly, small species of zooplankton such as *Diacyclops thomasi*, small *Daphnia* spp., *Ceriodaphnia* spp, and *Bosmina longirostris* are often important components of the zooplankton assemblage of lakes that have fish both in our study area and elsewhere (Brooks and Dodson 1965; Evans 1986; Elser et al. 1995). However, we could not confirm the absence of chaoborids in all fish-inhabited lakes owing to their migratory behaviour and our logistical constraints that did not allow us to conduct night sampling.

Table 2. The percentage of lakes with and without fish in which zooplankton taxa were numerically dominant.

Dominant species	Length[a] (mm)	No fish (never stocked) ($n = 5$)	Catostomidae, Cyprinidae, and Salmonidae ($n = 7$)	Salmonidae stocked annually ($n = 8$)	No fish, number of years since last stocked: 8–12 years ($n = 11$)	No fish, number of years since last stocked: 23–30 years ($n = 3$)
Copepoda[b]						
Diaptomus leptopus	1.3–2.5	100	0	37	46	67
Acanthodiaptomus denticornis	1.5–1.9	0	0	25	18	33
Acanthocyclops vernalis	0.8–1.8	0	0	13	36	0
Diacyclops thomasi	0.8–1.3	0	57	13	0	0
Orthocyclops modestus	0.8–1.3	0	29	13	0	0
Macrocyclops albidus	0.8–2.5	0	14	0	0	0
Eucyclops agilis		0	0	0	0	0
Diaptomus tyrelli	1.1–1.9	0	0	0	0	0
Cladocera[b]						
Daphnia (large species)[c]	1.3–2.3	80	29	25	82	33
Diaphansoma spp.	0.8–1.2	20	0	0	18	67
Ceriodaphnia spp.	0.4–1.0	0	13	37	0	0
Bosmina longirostris	0.5	0	29	13	0	0
Daphnia rosea	1.2–1.6	0	29	25	0	0
Chydorus sphaericus	0.4–0.7	0	0	0	0	0
Polyphemus pediculus	1.4	0	0	0	0	0
Alona spp.	0.3–0.5	0	0	0	0	0
Chaoboridae						
Chaoborus	8.0–10.2	100	29	38	100	100
Mean number of copepods		2.2	2.7	2.3	2.8	2.5
Mean number of cladocerans		2.2	4.3	3.3	2.5	2.5
Mean number of chaoborids		1.2	0.3	0.4	1.4	1.7
Mean total number of taxa		5.6	7.3	6.0	6.7	6.7

[a] From Edmondson 1959, Anderson 1968, Anderson and Fabris 1970, and our unpublished data.

[b] Rare species, those present in <5 lakes not included (*Diaptomus sicilis*, *Alonella nana*, *Acroperus harpae*, *Leptodora kindtii*, and *Graptoleberis testudinaria*).

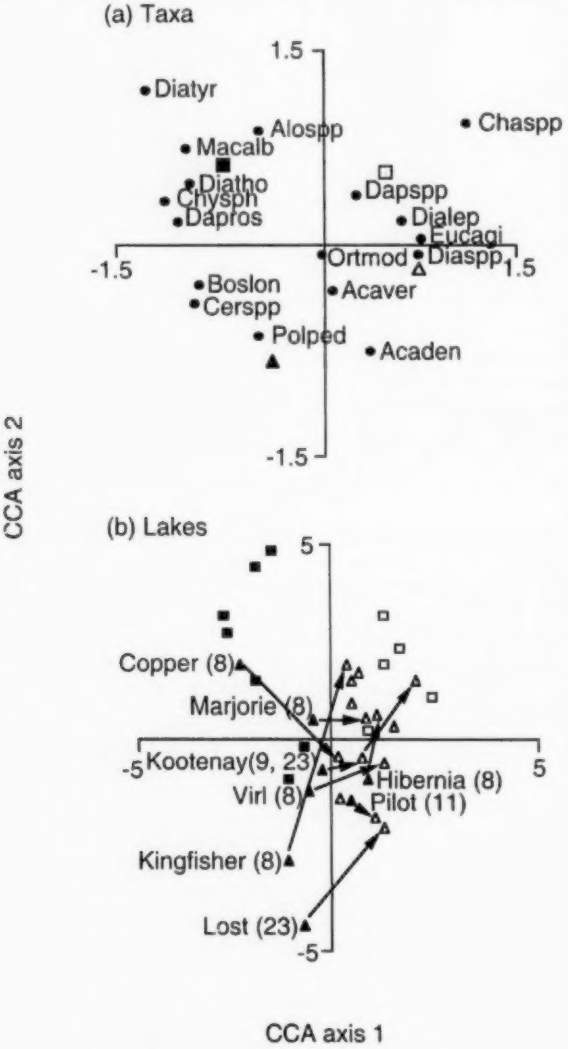
[c] *D. pulex*, *D. pulicaria*, and *D. schodeleri*.

Table 3. Mean abundances (individuals·L^{-1}) of zooplankton taxa used in CCA with data arranged into four lake categories according to their fish status at the time of sampling.

Taxon	Fishless, never stocked ($n = 5$)	Catostomidae, Cyprinidae, and Salmonidae ($n = 7$)	Salmonid stocked annually ($n = 8$)	Fishless, previously stocked ($n = 14$)[a]
Diaptomus leptopus	4.52	0.32	8.31	6.34
Acanthodiaptomus denticornis	0	0	4.19	2.13
Diaptomus tyrelli	0	0.11	0	0
Acanthocyclops vernalis	0.04	1.52	14.93	6.11
Diacyclops thomasi	0.11	7.38	6.25	0.17
Orthocyclops modestus	0	1.60	6.35	2.09
Macrocyclops albidus	0	0.06	0.01	0.01
Eucyclops agilis	0	0.01	0	0.11
Daphnia (large species)	5.24	5.41	13.16	3.71
Diaphanosoma	2.35	0.08	0.91	3.01
Ceriodaphnia	0	4.59	12.51	0.01
Bosmina longirostris	0	2.93	7.55	0.02
Daphnia rosea	0	9.17	7.96	0.01
Chydorus sphaericus	0.01	0.17	0.06	0.01
Polyphemus pediculus	0.03	0.03	1.25	0.01
Alona	0	0.03	0.01	0.01
Chaoborus	0.49	0.02	0.04	0.16

[a] Kootenay Pond was sampled in 1981 and 1996, thus $n = 14$.

Fig. 2. Association of zooplankton species, mountain lakes, and fish-stocking histories of lakes based on a CCA of biological survey data from 25 mountain lakes sampled on 34 occasions. (*a*) Zooplankton taxa and centroids of the 4 categories of fish-stocking histories. Fish-stocking histories are denoted by the following symbols: non-stocked lakes with complex fish assemblage (■), salmonid-stocked lakes (▲), fishless lakes previously stocked with salmonids (△), and fishless lakes that were never stocked with fish (□). Taxa are represented by six-letter taxonomic codes (full Latin binomials appear in Table 3). (*b*) Lakes. Linear chi-squared distance vectors represent recovery trajectories for lakes that had been frequently stocked with salmonids (▲) and thereafter returned to a fishless state (△) once salmonid stocking was stopped. Numbers in brackets show the number of years since a lake had been stocked with salmonids for the trajectories.



Other whole-lake biomanipulation experiments suggest that time scales of one to two decades may be required before zooplankton assemblages become stable. In agreement, Wissel and Benndorf (1998) found that zooplankton species changed for 15 years in a lake when the fish predator was eliminated. Over this period, the dominant predator changed from a planktivorous fish (eliminated) to *Chaoborus flavicans* and then to *C. obscuripes*.

Certain dominant taxa in the fishless reference lakes were present in the stocked lakes and thus were not eliminated when salmonids were stocked. *Diaptomus leptopus* and large cladocerans were both present in 67% of the stocked lakes, with *D. leptopus* the dominant copepod in 37%. Because of the presence of these calanoid and cladoceran taxa, once fish stocking ceased the zooplankton assemblage would be positioned to recover to the type of assemblage found in a fishless environment. Our data also indicate that introduced salmonids did not reduce zooplankton species richness from that characteristic of pristine fishless lakes. A short-term study by Northcote et al. (1978) also indicated that salmonid introductions into fishless low-elevation lakes did not substantially impoverish crustacean plankton assemblages. Cutthroat trout and Dolly Varden (*Salvelinus malma*) were introduced into fishless lakes, and after 2 years, these salmonids had little effect on the number of crustacean species and their seasonal abundances. However, chaoborid abundance declined dramatically.

Rainbow trout and brook trout are not efficient planktivores, at least relative to some species such as alewife *Alosa* and lake herring *Coregonus*. These latter fish species have long slender gill rakers capable of filtering zooplankton from water. Trout are generalists, feeding on a variety of large benthic invertebrate species as well as plankton such as large individuals of *Daphnia*, *Diaptomus*, and *Eurycercus* (Neave and Bajkov 1929; Rawson and Elsey 1948; Donald and Anderson 1982). Inefficient planktivory by trout may have permitted medium- and large-sized plankters such as *D. leptopus* and *Daphnia* to persist in the presence of fish in some of the stocked montane lakes. Once trout were no longer stocked, predation on these larger species of plankton would decline and zooplankton populations could rapidly adjust to the fishless environment. When intense inter- and intra-annual sampling occurred, Arnott et al. (1998) found that individual lakes could support close to the total number of zooplankton species in a region, although most were very rare. Some of these rare species would be positioned to re-establish more abundant populations when fish were removed from the lakes in our study. Moreover, diapausing or resting eggs of crustaceans may have enabled some species to survive the impact of fish (Parker et al. 1996), especially in the five lakes that were stocked infrequently.

Recovery of extirpated species to the pristine condition would require recolonization. The rapid appearance of chaoborids after stocking ceased is not surprising. Adults of this family of flies (Diptera) could freely disperse to these lakes from nearby fishless ponds. Recolonization for crustacean species eliminated from stocked lakes, however, would be more problematic. The available mechanisms for colonization may be transport within the feathers and digestive systems of waterfowl and shorebirds (Proctor 1964; Daborn 1976) or perhaps, like amphipods, transport within the fur of semi-aquatic mammals (Peck 1975).

Large calanoid copepods (generally >2.5 mm) such as *Hesperodiaptomus arcticus* and *H. shoshone* were not found in the reference lakes, although one or both of these species are usually present in fishless subalpine and alpine lakes in our study area (Anderson 1974; Donald et al. 1994; McNaught et al. 1999). *Hesperodiaptomus* and *Chaoborus* rarely occur together in mountain lakes (Anderson and Raasveldt 1974; Anderson 1980). *Chaoborus* are most common in montane and lower subalpine ecozones (Lamontagne et al. 1994). Perhaps this disjunct distribution of *Hesperodiaptomus* and *Chaoborus* is due to competitive exclusion because both genera prey on a similar size range of zooplantkon species (Anderson 1970; Dodson 1970; Anderson and Raasveldt 1974). *Hesperodiaptomus* may have a competitive advantage in cold alpine lakes, *Chaoborus*, in relatively warm montane lakes.

Within three decades of the last year of fish stocking, all 13 fishless lakes supported chaoborids, and in six, the dominant species of cladocerans and copepods were characteristic of reference lakes. This indicates that zooplankton assemblages can recover to near their pristine condition within a few decades after fish stocking ceases and after five decades of exposure to introduced fish populations (e.g., Hibernia and Marjorie). For some lakes, however, many decades may be required for some species to re-establish their former presence and position in the zooplankton assemblage. Twenty-three years after fish were last stocked in Lost Lake, the dominant copepod in the zooplankton assemblage was *Acanthodiaptomus denticornis*, which was not present in reference lakes. For some lakes, resilience of zooplankton communities to recover from the effects of introduced sport fish may be limited. Absence of interlake connectedness or low regional zooplankton species diversity may have reduced opportunities for recolonization. Thus, the impact of long-term fish stocking on zooplankton assemblages in some lakes may be irreversible at least in terms of time scales that may be half a century or more. However, our samples from only five reference lakes may have under represented the diversity of zooplankton assemblages in pristine fishless lakes. It is possible that *Acanthodiaptomus denticornis*, a relatively small species, was the original dominant calanoid in 3 of the 13 fishless lakes, and that populations of this species were relatively unaffected by the introduction of salmonids. In at least one fishless pond near our study area that would never have been stocked with fish, *A. denticornis* was present at mean densities of 4.2 L^{-1} in June, indicating that this species can be abundant in fishless habitats (Donald 1971). In Norway, *A. denticornis* occurred in lakes with and without fish and was not identified as a species negatively affected by fish (Langeland 1982).

Although zooplankton are considered to be highly effective dispersers over vast distances (e.g., >100 km) of low-relief terrain (Shurin et al. 2000), physical dispersal barriers (e.g., mountain ranges) may impede movement of microcrustaceans between lakes in mountainous landscapes. As a consequence, biological recovery of mountain lakes from the influence of sport-fish introductions can be expected to be slow. For example, McNaught et al. (1999) reported that zooplankton in an alpine lake had not yet recovered from the effects of introduced trout after 10 years despite the aided reintroduction of an ecologically important keystone species (*Hesperodiaptomus arcticus*). In general, our findings suggest that zooplankton assemblages in mountain lakes require on average 19 years to recover once fish stocking has been stopped.

Acknowledgements

This project was supported by Environment Canada, Heritage Canada, and Dr. Peter Leavitt of the University of Regina. We also appreciate the assistance of all those who collected zooplankton samples and determined fish community composition, especially David Mayhood, Bob Saunders, David Alger, Derek Donald, Teslin Donald, and Susanna Donald.

References

Anderson, R.S. 1968. The zooplankton of five small mountain lakes in south-western Alberta. Natl. Mus. Can. Nat. Hist. Pap. **39**: 1–19.

Anderson, R.S. 1970. Predator–prey relationships and predation rates for crustacean zooplankters from some lakes in western Canada. Can. J. Zool. **48**: 1229–1240.

Anderson, R.S. 1972. Zooplankton composition and change in an alpine lake. Verh. Int. Verein. Limnol. **18**: 264–268.

Anderson, R.S. 1974. Crustacean plankton communities of 340 lakes and ponds in and near the national parks of the Canadian Rocky mountains. J. Fish. Res. Board Can. **31**: 855–869.

Anderson, R.S. 1980. Relationships between trout and invertebrate species as predators and the structure of the crustacean and rotiferan plankton in mountain lakes. *In* Evolution and ecology of zooplankton communities. *Edited by* W.C. Kerfoot. Unversity Press of New England. Hanover, N.H. pp. 635–641.

Anderson, R.S., and Fabris, G. 1970. A new species of diaptomid copepod from Saskatchewan with notes on the crustacean community of the pond. Can. J. Zool. **48**: 49–54.

Anderson, R.S., and Raasveldt, L.G. 1974. *Gammarus* predation and the possible effects of *Gammarus* and *Chaoborus* feeding on the zooplankton composition in some small lakes and ponds in western Canada. Can. Wildl. Serv. Occas. Pap. 18.

Anonymous. 1994. Major ions and nutrients, method 01-1700, analysis of residue in surface, ground, and wastewater using gravimetric measurement. *In* Manual of analytical methods. Vol. 1. National Laboratory for Environmental Testing, Environment Canada, Burlington, Ont.

Arnott, S.E., Magnuson, J.J., and Yan, N.D. 1998. Crustacean zooplankton species richness: single- and multiple-year estimates. Can. J. Fish. Aquat. Sci. **55**: 1573–1582.

Bradford, D.F., Cooper, S.D., Jenkins, T.M., Kratz, K., Sarnelle, O., and Brown, A.D. 1998. Influences of natural acidity and introduced fish on faunal assemblages in California alpine lakes. Can. J. Fish. Aquat. Sci. **55**: 2478–2491.

Brooks, J.L., and Dodson, S.I. 1965. Predation, body size, and composition of plankton. Science (Washington, D.C.), **150**: 28–35.

Daborn, G.R. 1976. Colonization of isolated aquatic habitats. Can. Field-Nat. **90**: 56–57.

Dodson, S.I. 1970. Complementary feeding niches sustained by size-selective predation. Limnol. Oceanogr. **15**: 131–137.

Donald, D.B. 1971. The limnology and plankton of three temporary ponds in Alberta. M.Sc. thesis, University of Calgary, Calgary, Alta.

Donald, D.B. 1987. Assessment of the outcome of eight decades of trout stocking in the mountain national parks, Canada. N. Am. J. Fish. Manag. **7**: 545–553.

Donald, D.B., and Alger, D.J. 1986. Dynamics of unexploited and lightly exploited populations of rainbow trout (*Salmo gairdneri*) from coastal, montane, and subalpine lakes in western Canada. Can. J. Fish. Aquat. Sci. **43**: 1733–1741.

Donald, D.B., and Anderson, R.S. 1982. Importance of environment and stocked density for growth of rainbow trout in mountain lakes. Trans. Am. Fish. Soc. **111**: 675–680.

Donald, D.B., Anderson, R.S., and Mayhood, D.W. 1980. Correlations between brook trout growth and environmental variables for mountain lakes in Alberta. Trans. Am. Fish. Soc. **109**: 603–610.

Donald, D.B., Anderson, R.S., and Mayhood, D.W. 1994. Coexistence of fish and large *Hesperodiaptomus* species (Crustacea: Calanoida) in subalpine and alpine lakes. Can. J. Zool. **72**: 259–261.

Edmondson, W.T. 1959. Freshwater biology. 2nd ed. John Wiley and Sons, Inc., New York.

Elser, J.J., Luecke, C., Brett, M.T., and Goldman, C.R. 1995. Effects of food web compensation after manipulation of rainbow trout in an oligotrophic lake. Ecology, **76**: 52–69.

Evans, M.S. 1986. Recent major declines in zooplankton populations in the inshore region of Lake Michigan: probable causes and implications. Can. J. Fish. Aquat. Sci. **43**: 154–159.

Lamontagne, S., Donald, D.B., and Schindler, D.W. 1994. The distribution of four *Chaoborus* species (Diptera: Chaoboridae) along an elevation gradient in Canadian Rocky Mountain lakes. Can. J. Zool. **72**: 1531–1537.

Langeland, A. 1982. Interactions between zooplankton and fish in a fertilized lake. Holarct. Ecol. **5**: 273–310.

McNaught, A.S., Schindler, D.W., Parker, B.R., Paul, A.J., Anderson, R.S., Donald, D.B., and Agbeti, M. 1999. Restoration of the food web of an alpine lake following fish stocking. Limnol. Oceanogr. **44**: 127–136.

Mittelbach, G.G., Turner, A.M., Hall, D.J., Rettig, J.E., and Osenberg, C.W. 1995. Perturbation and resilience: a long-term whole-lake study of predator extinction and reintroduction. Ecology, **76**: 2347–2360.

Neave, F., and Bajkov, A. 1929. Reports of the Jasper Park lakes investigations, 1925–26. V. Food and growth of Jasper Park fishes. Contri. Can. Biol. Fish. New Series, **4**: 197–219.

Northcote, T.G. 1988. Fish in the structure and function of freshwater ecosystems: a "top-down" view. Can. J. Fish. Aquat. Sci. **45**: 361–379.

Northcote, T.G., Walters, C.J., and Hume, J.M.B. 1978. Initial impacts of experimental fish introductions on the macrozooplankton of small oligotrophic lakes. Verh. Int. Verein. Limnol. **20**: 2003–2012.

Parker, B.R., Wilhelm, F.M., and Schindler, D.W. 1996. Recovery of *Hesperodiaptomus arcticus* populations from diapausing eggs following elimination by stocked salmonids. Can. J. Zool. **74**: 1292–1297.

Peck, S.B. 1975. Amphipod dispersal in the fur of aquatic mammals. Can. Field-Nat. **89**: 181–182.

Proctor, V.W. 1964. Viability of crustacean eggs recovered from ducks. Ecology, **45**: 656–658.

Rawson, D.S., and Elsey, C.A. 1948. Reduction in the longnose sucker population of Pyramid Lake, Alberta, in an attempt to improve angling. Trans. Am. Fish. Soc. **78**: 13–31.

Shurin, J.B., Havel, J.E., Leibold, M.A., and Pinel-Alloul, B. 2000. Local and regional zooplankton species richness: a scale-independent test for saturation. Ecology, **81**: 3062–3073.

ter Braak, C.J.F. 1991. CANOCO—a FORTRAN program for canonical community ordination by partial detrended canonical correspondence analysis, principal components analysis, and redundancy analysis. Agricultural Mathematics Group, P.O. Box 100, 6700 AC, Wageningen, the Netherlands.

Vinebrooke, R.D., and Graham, M.D. 1997. Periphyton assemblages as indicators of recovery in acidified Canadian Shield lakes. Can. J. Fish. Aquat. Sci. **54**: 1557–1568.

von Ende, C.N. 1979. Fish predation, interspecific predation, and the distribution of two *Chaoborus* species. Ecology, **60**: 119–128.

Wissel, B, and Benndorf, J. 1998. Contrasting effects of the invertebrate predator *Chaoborus obscuripes* and planktivorous fish on plankton communities of a long-term biomanipulation experiment. Arch. Hydrobiol. **143**: 129–146.

Climate regimes and water temperature changes in the Columbia River: bioenergetic implications for predators of juvenile salmon

James H. Petersen and James F. Kitchell

Abstract: We examined how climatic regime shifts may have affected predation rates on juvenile Pacific salmonids (*Oncorhynchus* spp.) by northern squawfish (*Ptychocheilus oregonensis*, also called northern pikeminnow), smallmouth bass (*Micropterus dolomieu*), and walleye (*Stizostedion vitreum*) in the Columbia River. During 1933–1996, oceanic, coastal, and freshwater indices of climate were highly correlated, and an index for the Columbia River Basin suggested that climate shifts may have occurred about 1946, 1958, 1969, and 1977. Summer water temperature varied as much as 2°C between climate periods. We used a bioenergetics model for northern squawfish, the most important piscivore, to predict that predation on salmonids would have been 26–31% higher during two periods with relatively warm spring–summer water temperatures (1933–1946, 1978–1996) than during an extremely cold period (1947–1958). Predicted predation rates of northern squawfish were 68–96% higher in the warmest year compared with the coldest year. Predation rates of smallmouth bass and walleye on juvenile salmonids varied among climate periods similar to rates predicted for northern squawfish. Climatic effects need to be understood in both freshwater and nearshore marine habitats, since growth rates of salmon populations are especially sensitive to mortality during early life stages.

Résumé : Nous avons examiné comment les changements de régime climatique peuvent avoir affecté les taux de prédation des jeunes saumons du Pacifique (*Oncorhynchus* spp.) par la Sauvagesse du Nord (*Ptychocheilus oregonensis*, aussi appelé le Méné-brocheton du Nord), l'Achigan à petite bouche (*Micropterus dolomieu*) et le Doré jaune (*Stizostedion vitreum*) dans le Columbia. De 1933 à 1996, les indices climatiques de l'océan, de la côte et des eaux douces montrent une forte corrélation et un indice calculé pour le bassin versant du Columbia laisse croire à l'existence de changements climatiques vers 1946, 1958, 1969 et 1977. Les températures estivales de l'eau ont subi des variations pouvant atteindre 2°C d'une période climatique à une autre. Un modèle bioénergétique a permis de prédire que, chez la Sauvagesse du Nord, le piscivore le plus important, la prédation des salmonidés aurait été de 26–31% plus élevée durant les deux périodes où les températures de printemps et d'été ont été relativement plus chaudes (1933–1946, 1978–1996) que pendant une période très froide (1947–1958). Les taux prédits de prédation sont de 68–96% plus élevés l'année la plus chaude que l'année la plus froide. La prédation exercée par les Achigans à petite bouche et les Dorés jaunes sur les salmonidés a varié d'une période à l'autre selon les taux prédits chez la Sauvagesse du Nord. Il est donc essentiel de comprendre les effets des changements climatiques, tant dans les eaux douces que dans les habitats marins côtiers, puisque les taux de croissance des populations de saumons sont particulièrement sensibles à la mortalité durant les premiers stades de vie.

[Traduit par la Rédaction]

Introduction

Salmon (*Oncorhynchus* spp.) returns to the Columbia River have recently been <2 million adults compared with returns of 8–16 million adults in the early 1800s (National Research Council 1996). Throughout the Pacific Northwest, salmonid species have suffered similar declines (Nehlsen et al. 1991) and 17 stocks have been listed as threatened or endangered under the Endangered Species Act (http://www.nwr.noaa.gov/). The causes for these declines are often ascribed to human activities, including overharvest, habitat destruction from various land-use practices, and construction of dams throughout the basin. Numerous studies have, however, described how natural changes in the ocean environment may have contributed to declining salmon populations in the Columbia River and other basins, especially during the last 70 years (e.g., Francis and Hare 1994; Mantua et al. 1997; Beamish et al. 1999*b*).

Natural oscillations in climate and ocean conditions in the North Pacific Ocean have occurred on time scales of 2–7 and 20–30 years, and a poorly resolved 50- to 75-year period (Ware 1995). Short-term changes are associated with the El Niño/Southern Oscillation phenomenon, while the 20- to 30-year pattern has been called the Pacific Decadal Oscillation (PDO) (Mantua et al. 1997) or climate "regimes" (Beamish et al. 1999*b*). The PDO in particular has been correlated with temporal and latitudinal changes in salmon production,

Received September 1, 2000. Accepted April 27, 2001. Published on the NRC Research Press Web site at http://cjfas.nrc.ca on August 27, 2001.
J15953

J.H. Petersen.[1] U.S. Geological Survey, Western Fisheries Research Center, Columbia River Research Laboratory, Cook, WA 98605, U.S.A.
J.F. Kitchell. Center for Limnology, University of Wisconsin, Madison, WI 53706, U.S.A.

[1] Corresponding author (e-mail: jim_petersen@usgs.gov).

DOI: 10.1139/cjfas-58-8-1831

with salmon catch in the Pacific Northwest being higher during periods when the PDO is negative and lower during periods with positive PDO (Francis and Hare 1994; Mantua et al. 1997). The salmon catch in Alaska has tended to be out of phase with the catch in the Pacific Northwest (Hare et al. 1999). Timing of ocean entry, competition for food, temperature-limited growth, and predation on juveniles are some of the mechanisms regulating salmon production that might be affected by ocean conditions and climate oscillation (Pearcy 1992).

Climate oscillations in the North Pacific Ocean influence not only the ocean environment but also force changes in weather patterns throughout western North America. Stream discharge, stream flow timing, and snowpack, for example, are well correlated over much of the Pacific Northwest, and such environmental variables are driven by large-scale atmospheric patterns (Cayan and Peterson 1989; Cayan et al. 1999). Cayan and Peterson (1989) found that stream flows in the Pacific Northwest were correlated with positive sea level pressure anomalies over the central North Pacific Ocean. Precipitation patterns in western North America are linked to decadal changes in atmospheric circulation and sea surface temperature anomalies on scales that range from regional to global (Cayan et al. 1998). The PDO and April–September flow on the lower Columbia River have been well correlated for 1900–1997 (Hamlet and Lettenmaier 1999), even though dams have influenced flows in the lower Columbia River since the late 1930s.

In this paper, we examined how climate and water temperature in the Columbia River varied between 1933 and 1996 and how temperature changes may have influenced predation rates on juvenile salmonids. Predation occurs throughout the mainstem Columbia and Snake rivers, with especially high losses occurring near dams and in the lower Columbia River (Ward et al. 1995; Beamesderfer et al. 1996). The major piscivores are northern squawfish (*Ptychocheilus oregonensis*, also called northern pikeminnow), smallmouth bass (*Micropterus dolomieu*), walleye (*Stizostedion vitreum*), and channel catfish (*Ictalurus punctatus*) (Rieman et al. 1991; Vigg et al. 1991), with only northern squawfish being native to the Columbia River Basin. Northern squawfish, smallmouth bass, and walleye have been estimated to consume 7–17% of all salmonids that annually migrate through John Day Reservoir on the Columbia River (Petersen 1994), and northern squawfish alone may consume 16 million juvenile salmonids annually, 8% of the annual smolt outmigration (Beamesderfer et al. 1996).

Materials and methods

First, we compared three indices of climate to define periods of consistent climate conditions for the Columbia River Basin. Second, we partitioned and tested the differences in water temperature based on the observed climate periods. Finally, we conducted bioenergetic simulations to estimate the predation potential by northern squawfish and other piscivores under different climate and water temperature regimes.

Climate periods or regimes in the Columbia River Basin

Three indices of climate conditions were compared, the PDO index (Mantua et al. 1997), the Pacific Northwest Index (PNI) (Ebbesmeyer and Strickland 1995), and an index specific to the Columbia River Basin, which we call the Columbia Basin Index (CBI). The PDO is a normalized index based on North Pacific sea surface temperature (Mantua et al. 1997). The PDO was downloaded on May 22, 2000 from web site http://www.iphc.washington.edu/staff/hare/html/decadal/post1977/pdo1.html. The PDO has been shown to correlate well with several other indices of climate and adult salmon landings in the North Pacific Ocean (Mantua et al. 1997). The PNI combines air temperature at Olga in the San Juan Islands, total precipitation at Cedar Lake in the Cascade Mountains, and snowpack depth on Mount Rainier. PNI values were obtained on April 28, 2000 from web site http://www.cqs.washington.edu/data/pni_ascii.html.

Because neither the PNI nor the PDO was developed specifically for the Columbia River Basin, we derived an index based on unregulated stream flows within the Basin. We used data from the Hydro-Climatic Data Network (HCDN) (Slack and Landwehr 1992) to derive a CBI of regional climate. The HCDN includes U.S. Geological Survey data from streams and rivers that have not been influenced by watercourse regulation (dams, etc.), irrigation diversions, groundwater pumpage, or land-use change (Slack and Landwehr 1992). Gage records used were within the boundary of the Columbia River Basin, covered 1938–1999, and had <3 years of missing data for an individual gage. Only one gage record was used per watershed (minimum drainage area 1000 km^2).

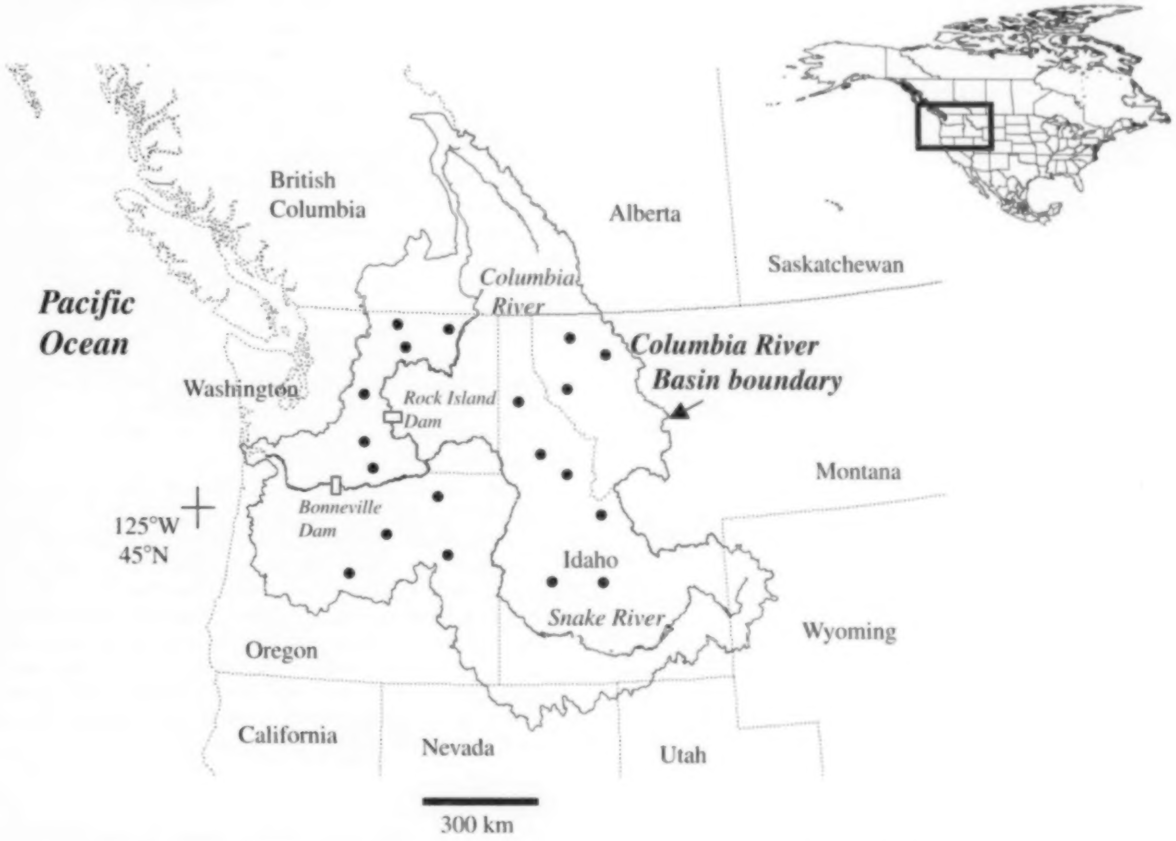
The CBI was the average of normalized flow ((monthly average – long-term average)/standard deviation of the long-term average) across all 19 selected stations (Fig. 1) in the Columbia River Basin for a particular month. Principal components analysis of normalized discharge was used to evaluate whether there were intrabasin patterns among stations. Smith (2000) used a similar protocol to standardize stream flows in British Columbia. Climate periods were identified by examining time series of the PDO, PNI, and CBI (May–August indices). Shifts from positive to negative or vice versa, and a consistent index for periods of >5 years, would suggest a significant change in Basin climate. We also made cumulative sum plots of the CBI (CuSum), which assist in identifying shifts in time series of climatic indices (Beamish et al. 1999*b*).

Water temperature in the Columbia River

For bioenergetics modeling and related hypothesis testing, we used long-term temperature series collected at Bonneville Dam (1938–1996) and Rock Island Dam (1933–1996) on the Columbia River (Fig. 1). These are the two longest time series of water temperature available for the Columbia River (www.streamnet.org), and they represent an upper and a lower river site. Water temperature was monitored in the dam forebays near turbine intakes (scrollcase); temperature measurements were taken at ~14 m at Bonneville Dam and ~5 m at Rock Island Dam. We assumed that the Rock Island Dam temperatures characterized the upper reaches of the Columbia River (Quinn et al. 1997). Bonneville Dam (river mile 154.5) temperatures were biased high when compared with temperatures measured at Warrendale below the dam (river mile 141.0, U.S. Geological Survey Station No. 14128910, average difference +0.6°C across all months, 1978–1991). We therefore adjusted the long-term Bonneville Dam record for each date between 1938 and 1996 using the average monthly difference between Bonneville Dam and the Warrendale gage.

To test for climate period and location (Bonneville versus Rock Island) effects, we divided water temperatures into three "seasons": April 1 – May 31, June 1 – August 15, and August 16 – October 31. The April 1 – May 31 season corresponds to increasing flow, slowly increasing water temperature, and outmigration of juvenile sockeye salmon (*Oncorhynchus nerka*), spring chinook salmon (*Oncorhynchus tshawytscha*), and steelhead (*Oncorhynchus mykiss*) in the Columbia River. The June 1 – August 15 period corresponds to decreasing flow, rapidly increasing water temperature, and outmigration of fall chinook salmon. The August 16 – October 31 pe-

Fig. 1. Columbia River Basin and location of climate index and temperature data sites (Bonneville and Rock Island dams) on the Columbia River. Nineteen HCDN stream flow gages (●) were used to compute the CBI of climate. The Columbia and Snake rivers are shown as heavy lines.



riod corresponds to peak and declining temperature, low flows, and few juvenile salmonids in the system.

Analysis of variance (ANOVA) tests of temperature change were conducted on daily anomalies, the differences between a daily temperature and the long-term average temperature on that day and location. Use of temperature anomalies removed trends present within a season, and tests focused on deviations from the long-term average. If a significant model effect was detected ($P < 0.05$), Tukey's multiple range test was used to compare means.

Bioenergetic simulations

Bioenergetics modeling is a proven tool for studying predation rates, temperature effects, and other processes (Kitchell et al. 1977). The model that we applied uses a mass balance approach where growth (G) is equal to consumption (C) minus respiration (R) and excretory losses (E): $G = C - R - E$. Individual terms on the right side of the equation are expanded based primarily on fish size, activity, and water temperature. Fish in different habitats can be modeled by varying diets, while food availability is adjusted with a fit parameter, called a p value, which is the proportion of maximum ration. The sensitivity, merits, and constraints of bioenergetics models have been examined in detail (e.g., Bartell et al. 1986). Bioenergetics models have been shown to be especially useful for evaluating variations in temperature (Shuter and Post 1990).

For northern squawfish, predation was simulated using the bioenergetics model, parameters, and assumptions described in Petersen and Ward (1999). We modeled an individual fish that was 8 years old, had a starting mass of 668 g, and grew between April 1 and October 31. Northern squawfish that consume juvenile salmonids range in size from ~200 g (~250 mm fork length, age ~5 years) to >2000 g (>500 mm fork length, age 15 years or older), and an 8-year-old fish represents a medium-sized predator in the population. Petersen and Ward (1999) showed that 55% (21 of 38) of the size–frequency distributions of northern squawfish in John Day Reservoir were normally distributed and that populations with slightly skewed weight distributions could be modeled reasonably well with one size-class.

Because we had long-term temperatures from two locations in the Columbia River, Bonneville Dam and Rock Island Dam, we modeled fish representing two general reaches, upper versus lower river. We varied diet to model three types of habitat: a midreservoir habitat, a near-dam habitat (tailrace or forebay), and the free-flowing portion of the Columbia River below Bonneville Dam. Various studies have shown how northern squawfish diets vary among these types of habitat (e.g., Poe et al. 1991). The diet of predators in the free-flowing habitat below Bonneville Dam was based on collections made in that reach, while diets for the other two habitats derive from John Day Reservoir studies (Poe et al. 1991; Petersen and Ward 1999). Although forebay and tailrace near-dam habitats are physically dissimilar, these areas were combined in model runs, since predators in both of these habitats have a relatively high percentage of juvenile salmonids in their diet

(Petersen and Ward 1999). Daily temperature in all simulations was interpolated from average monthly temperature for a particular period.

Swimming speeds in the three habitat types were 1.3 cm·s^{-1} (free-flowing and midreservoir habitats) and 7.0 cm·s^{-1} (near-dam habitat). The near-dam speed is a compromise of speeds applied by Petersen and Ward (1999): 9.2 cm·s^{-1} for a tailrace and 5.0 cm·s^{-1} for a forebay. Swimming speeds are based on studies of radio-tagged northern squawfish in the Columbia and Snake rivers (Martinelli and Shively 1997; J.H. Petersen, unpublished data).

We do not know how prey availability and predator diets might have changed during the last 70 years in the Columbia River, so we used two alternative assumptions for modeling growth. First, we assumed that predator growth increments in the past were the same as recent increments or that predators would adjust their food intake to achieve a fixed growth. For these simulations, the predator started at 668 g and grew to 813 g during the season. In a comparable set of simulations, we assumed fixed food availability and allowed the growth rate of the predator to vary. We fit the model p value for the first climate period (1933–1946, see Results) in each reach habitat simulation and used this p value for the other periods. With this approach, consumption of salmonids was the same during the earliest climate period for both the fixed growth and the fixed food availability simulations in a reach habitat.

Variation in stream flow, precipitation, snowmelt, and temperature between years may be considerable depending on short-term phenomena such as El Niño events (Cayan et al. 1999). Using the average daily temperature in July, we determined the warmest and coolest year for the upper and lower reaches and compared cumulative consumption of juvenile salmonids in near-dam habitats between these extreme years.

Finally, we compared salmonid consumption between northern squawfish and two other important piscivores in the system, smallmouth bass and walleye (Vigg et al. 1991), using the bioenergetic formulations and parameters of Roell and Orth (1993) (smallmouth bass) and of Kitchell et al. (1977) (walleye). We used diet summaries for all three predators in John Day Reservoir (Poe et al. 1994) and applied temperatures from both Rock Island and Bonneville dams. All piscivores in these simulations grew from 668 to 813 g between April 1 and October 31. Simulations for each predator species included a loss in mass during spawning.

Results

Climate periods in the Columbia River Basin

Average discharge at selected gages in the Columbia River Basin ranged from 62 to 2472 m^3·s^{-1}. Smaller drainage basins had somewhat high variations in flows, as expected. Principal components analyses of normalized flows for May, June, July, and August showed relatively high correlations among gaging stations, with no obvious outliers when stations were plotted on principal components axes. The first two eigenvalues explained from 76% (May) to 82% (July) of the total variation in normalized flows. We retained all 19 stations within the basin for index development.

Oceanic, coastal, and Columbia River Basin indices of climate varied in a similar fashion for the period 1933–1996, although there were some obvious differences after about 1980 (Fig. 2). The PNI and PDO were strongly correlated (Pearson's correlation coefficient (τ) = 0.69, $P < 0.001$, n = 64). For the months of May, June, July, and August, the CBI was negatively correlated with the PNI (τ = –0.42 to –0.67, $P < 0.001$, n = 64) and also with the PDO (τ = –0.27 to –0.48, $P < 0.04$, n = 64, years 1933–1996).

Time series of the indices showed distinct and coherent shifts between positive and negative values (Fig. 2), suggesting several concurrent climate changes in the North Pacific Ocean, the coastal zone, and the Columbia River Basin between 1933 and 1996. A strong shift in all indices occurred in 1945–1946, when the PNI and the PDO went from positive to negative and the CBI switched from negative to slightly positive (Fig. 2). The period of 1947–1958 had higher than average stream discharges in the Columbia River Basin during May–August. From about 1959 to 1968, the PNI was near zero, while the CBI varied from slightly negative (May) or positive (June) to mixed (July and August). From 1969 to 1977, the PNI was negative, suggesting cool, wet weather, while the CBIs were consistently positive, suggesting higher than average stream flows in spring through summer. In 1977, the PNI shifted dramatically to indicate warmer and dryer weather in the coastal areas of the Pacific Northwest (Fig. 2), while the CBI stream flow indices showed a more complex pattern. Normalized stream flows declined in most months during 1977–1980, increased in 1980–1984, decreased to below-average flows during 1985–1995, and then showed above-average flows in 1996–1999.

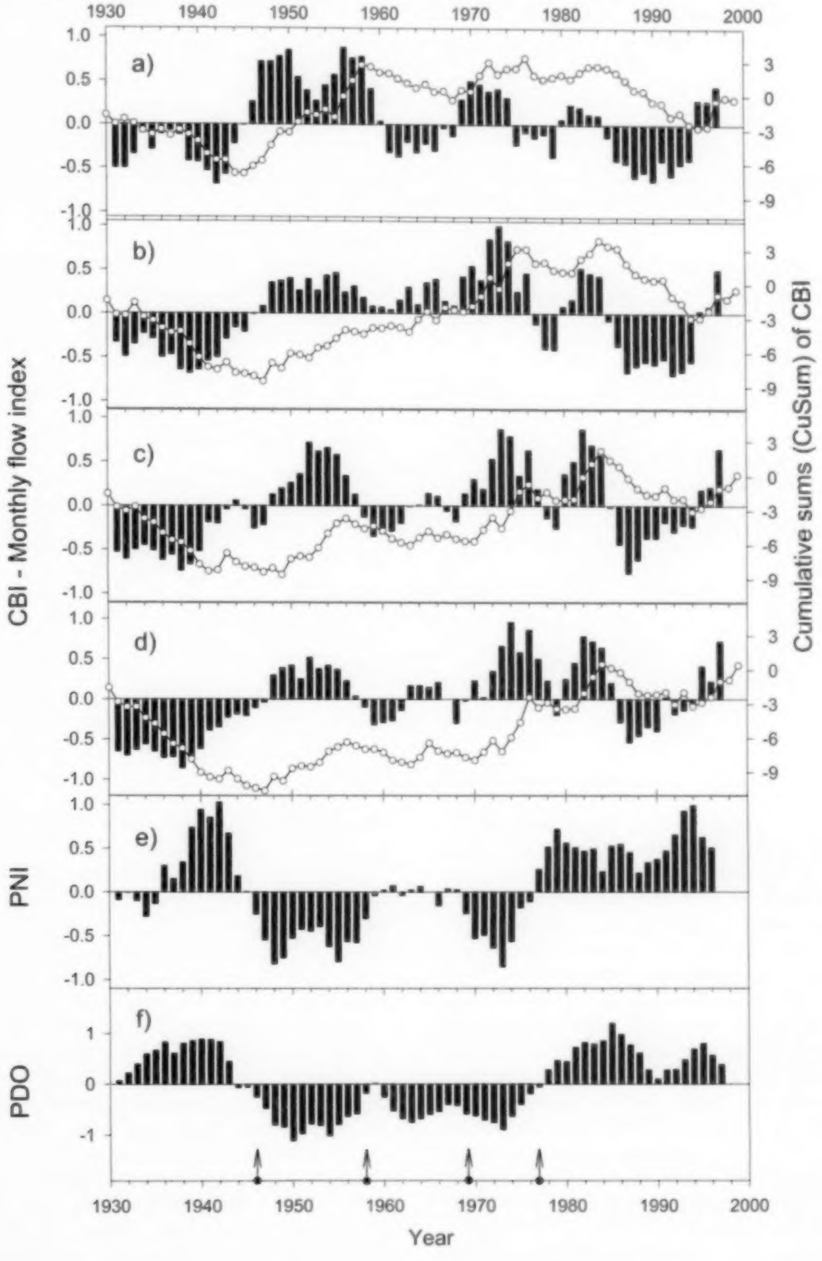
The patterns observed in climate indices (Fig. 2) between 1933 and 1999 suggested that there were roughly four times when climate conditions may have shifted from "high flows" to "low flows" in the Columbia River Basin. We used these four times to divide the overall period into five intervals for temperature analysis (Table 1). We recognize that there are several short-term variations in the stream flow indices that could further divide this period (especially for the years 1980–1999); however, we were interested primarily in longer (>5 years) climatic shifts that were also recognizable in the nearshore marine and ocean environments.

Water temperature

Average July water temperatures, shown as an indicator of short-term variability and long-term trends (Fig. 3), differed by as much as 2°C annually and as much as 4°C over a 4-year period (1955–1959). At both dams where temperatures were recorded from 1938 to 1996, water was relatively warm in the 1930s, cooler in the late 1940s to 1950s, and gradually warmed from about 1960 to 1996 (Fig. 3). The temperature difference between the upriver and downriver sites, Rock Island and Bonneville dams, was fairly consistent for July.

Temperature anomalies were significantly different among climate periods for each location-by-season test (six ANOVAs, all $P < 0.001$). Fifty-two of 60 pairwise comparisons were significant ($P < 0.05$, results not shown), suggesting considerable differences in water temperature among the climate periods within a season (Table 1). At Bonneville Dam, the pattern of temperature change was fairly consistent among the seasons: moderately warm temperatures during 1938–1946, coolest temperatures during 1947–1958, gradually warming temperatures in 1959–1977, and warmest temperatures for the period 1977–1996 (Table 1). Water temperature during April 1 – May 31 varied <1°C during the 58-year period, whereas from June 1 to August 15, temperature increased by as much as 2°C between the 1947–1958 period and the recent period (1977–1996). At Rock Island Dam, the pattern was slightly different (Table 1). For April 1 – May 31 and June 1 – August 15, water temperatures were about equal

Fig. 2. Climate indices for the (*a–d*) Columbia River Basin (CBI), (*e*) nearshore marine zone (PNI), and (*f*) North Pacific Ocean (PDO index). CBI plots are shown for (*a*) May, (*b*) June, (*c*) July, and (*d*) August. Vertical bars are 5-year running averages and line plots in the CBI panels are cumulative sums of yearly indices (CuSum). Vertical arrows along the bottom axis of Fig. 2*f* indicate times used to divide the 1933–1996 period for temperature testing and bioenergetic modeling (see text).



during the earliest period (1933–1946) and the latest period (1978–1996); in the intervening period (1947–1977), water temperature during April 1 – August 15 was ~1.0–1.5°C lower. During August 16 – October 30, water temperature remained fairly constant from 1933 to 1977 and then increased by ~1.0°C after 1977. Water temperature was higher during spring and summer seasons at Bonneville Dam than at Rock Island Dam, but fall season temperatures were about equal (Table 1).

Bioenergetic simulations

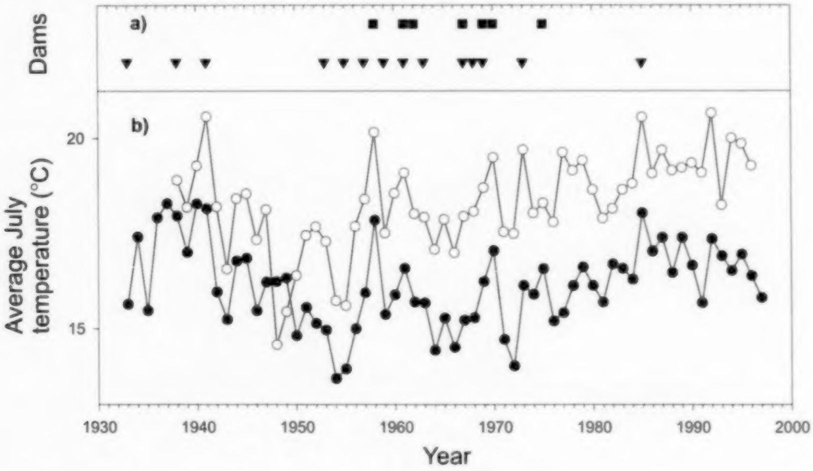
In simulations within a given reach and habitat, northern squawfish consumed the fewest salmonids during the 1947–1958 period, consumption of salmonids increased between

Table 1. Average daily water temperature (°C) at Bonneville Dam (1938–1996, adjusted temperatures) and at Rock Island Dam (1933–1996) on the Columbia River.

	Bonneville Dam (starts 1938)			Rock Island Dam (starts 1933)		
Period	Apr. 1 – May 31	June 1 – Aug. 15	Aug. 16 – Oct. 31	Apr. 1 – May 31	June 1 – Aug. 15	Aug. 16 – Oct. 31
1933 or 1938–1946	11.0	17.1	16.2	9.1	15.8	16.5
1947–1958	10.4	16.1	16.2	7.5	14.7	16.8
1959–1969	10.6	17.1	16.8	7.9	14.7	16.4
1970–1977	10.5	17.5	17.2	8.1	14.9	16.8
1978–1996	11.2	18.2	18.0	8.6	15.8	17.8

Note: Temperatures were averaged for three seasons and for five intervals that correspond to climate periods in the Columbia River Basin. Standard errors for all estimates are ~0.1°C.

Fig. 3. Average daily water temperature during July at Bonneville (○) and Rock Island (●) dams from 1933 to 1996. Also shown is the year of construction for dams on the lower Snake River (■) and Columbia River (▼, U.S. and Canadian dams).



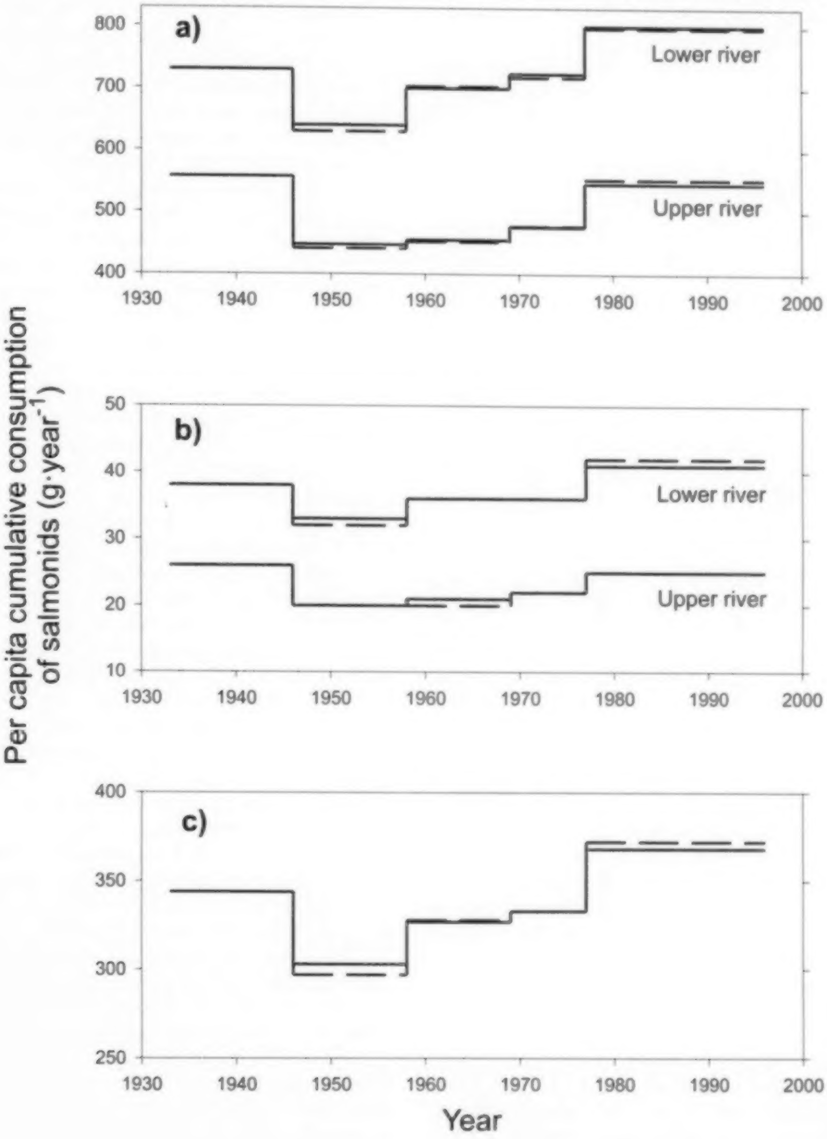
1959 and 1977, and the highest consumption of salmonids occurred either in the period prior to 1947 or in the period after 1977 (Fig. 4). In the upper river dam habitat (Fig. 4*a*), consumption of salmonids was about equal in the earliest and the latest period, while consumption of salmonids was ~26% lower during 1947–1958. In the lower river near-dam habitat, consumption of salmonids was highest in the 1978–1996 period, being ~9% higher than during 1933–1946 and ~27% higher than during the cool 1947–1958 period. In the midreservoir habitat (Fig. 4*b*), the trends were similar to the trends in the near-dam habitat just described. In the upper river, annual salmonid consumption by the simulated northern squawfish varied from a low of 20 g (1947–1970) to a high of 26 g (1933–1946). Consumption was similar in the earliest and latest periods (25 and 26 g). In the lower river, annual salmonid consumption varied from 32 g (1947–1958) to 42 g (1978–1996), a difference of ~31%.

In the free-flowing river habitat below Bonneville Dam (Fig. 4*c*), predation on salmonids was highest during the 1978–1996 period, being ~26% higher than during the coolest period (1947–1958). Predation on salmonids in this habitat during recent years was predicted to be ~8% higher than during 1933–1946. Allowing growth to vary (fixed food availability) did not produce qualitatively different results from the fixed-growth simulations (Fig. 4). Cumulative salmonid consumption differed by <5% between the two different types of simulations. Differences in cumulative annual consumption of salmonids between the habitats (Fig. 4) was caused by different diets used in the simulations and was not related to climate effects. For example, salmonids in northern squawfish were 28–60% of the diet below Bonneville Dam, 28–53% of the diet in near-dam simulations, and 3–9% of the diet for fish in the midreservoir habitat.

Comparisons of potential predation on juvenile salmonids during the coldest and the warmest years between 1933 and 1996 produced dramatic differences. Using Bonneville Dam temperatures, the simulated predator consumed 496 g during the coldest year (1948) and 974 g during the warmest year (1992) (+96%). Using Rock Island Dam temperatures, predation increased from 358 g of salmonids during the coolest year (1954) to 602 g during the warmest year (1937) (+68%).

The three piscivores had similar relative responses to changing water temperature (Fig. 5). Using Bonneville Dam temperatures, the lowest cumulative consumption of salmonids occurred during the 1947–1958 period and the highest consumption occurred during the 1978–1996 period (Fig. 5*a*). Using Rock Island Dam temperatures, the highest consumption occurred during 1933–1946 (Fig. 5*b*). Piscivore diet of salmonids varied from low (0–5%, smallmouth bass) to high (45–79%, northern squawfish), causing the mass of salmonids

Fig. 4. Cumulative salmonid consumption simulated for an 8-year-old northern squawfish in three habitats ((*a*) near-dam, (*b*) midreservoir, and (*c*) below Bonneville Dam) in the Columbia River during five climate periods (1933–1996). Near-dam and midreservoir habitats were simulated for temperature regimes in both the upper and the lower river. Simulations also varied according to assumptions about fixed growth (solid lines) or fixed food availability (dashed line) during this period (see text).



annually consumed to differ greatly between simulated piscivores: northern squawfish (543–649 g salmonids eaten downriver, 407–488 g upriver), smallmouth bass (9–10 g downriver, 7–8 g upriver), and walleye (121–136 g downriver, 104–115 g upriver).

Discussion

Studies on other species have shown how fish distributions, recruitment, or growth often correlates well with climate conditions and water temperature. Shuter and Post (1990), using bioenergetic arguments, concluded that the northern distributional limits of yellow perch (*Perca flavescens*) and Eurasian perch (*Perca fluviatilis*) are regulated by climate and may shift north with a warming climate. Elliott et al. (2000) observed a close correlation between the emergence timing of brown trout (*Salmo trutta*) and an index of climate from the North Atlantic Ocean. Walters and Ward (1998) suggested that declines in marine survival rates for steelhead, coho salmon (*Oncorhynchus kisutch*), chinook salmon, and Atlantic salmon (*Salmo salar*) may be related to climate changes, particularly increased exposure to ultraviolet radia-

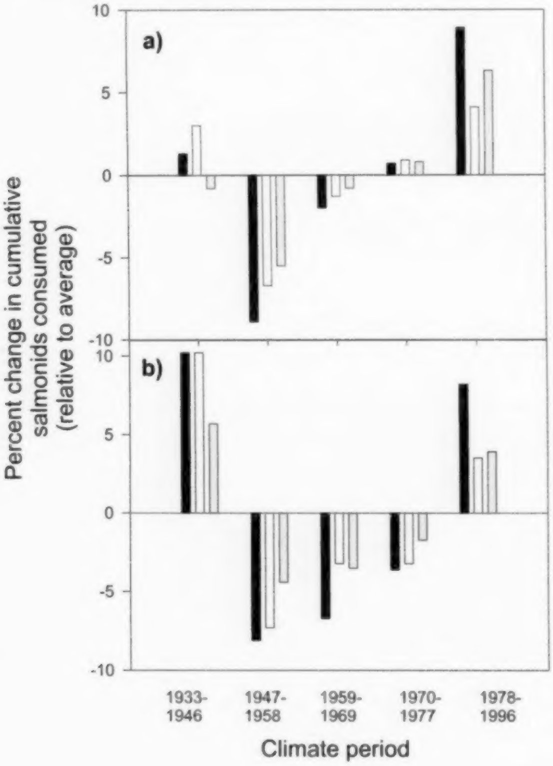


Fig. 5. Percent difference (relative to the overall average) in cumulative per capita consumption of salmonids in the Columbia River by northern squawfish (solid bars), smallmouth bass (open bars), and walleye (gray bars). Estimates were made using bioenergetics models and water temperature from the (*a*) Bonneville and (*b*) Rock Island dams.

tion during freshwater residence. Water temperature controls a wide variety of processes in the early life history of salmonids, such as days to hatch and emergence, feeding rate, and growth rate (e.g., Beacham and Murray 1990).

Our analyses suggested that large-scale climate oscillations, or regime shifts, have likely caused water temperature in the Columbia River to vary several degrees between 1933 and 1996. Since feeding by freshwater piscivores is temperature dependent, predation-related mortality of juvenile salmonids has probably varied due to natural climate change. Life table sensitivity analyses for chinook salmon suggested that increasing survival during the first year of life produces the largest increase in λ, the population growth rate (e.g., Emlen 1995). For seven index stocks of spring chinook salmon, a 10% reduction in mortality during the first year resulted in a 41.5% increase in λ (National Marine Fisheries Service 2000). Predation loss of juvenile salmonids in the mainstem Columbia and Snake rivers likely varies 25–30% between the warmest and coldest climate periods and by as much as 96% between the warmest and coldest years. Bradford (1995), in a review of Pacific salmon survival rates, concluded that both freshwater and marine habitats contribute roughly equally to the interannual variability in total mortality. Thus, the effects of climate change in freshwater may significantly alter first-year survival, with consequences for population growth rates, stock recovery, and management.

Unregulated stream flow, used for our basin index (CBI), has often been used to describe climate changes or trends (Cayan and Peterson 1989; Lins and Slack 1999). A major advantage of using the HCDN data for CBI was that indices were independent of dam construction, irrigation withdrawals, and other watercourse changes that have been extensive in the Columbia River Basin. Trends in the CBI should reflect differences in overall climate and processes regulating stream flow such as precipitation, air temperature, and snowmelt rather than human-caused changes. A second advantage of the CBI for our purposes is that water temperatures in streams and rivers are generally well correlated with flow (e.g., Quinn et al. 1997). June–July flow and temperature were negatively correlated at four dams on the Columbia and Snake rivers ($P < 0.001$; Quinn et al. 1997). Finally, stations used to compute the CBI were broadly distributed throughout the Columbia River Basin, whereas the PNI uses only three stations and characterizes conditions in the nearshore marine environment and on the western slope of the Cascade Mountain Range.

The primary shifts in climate conditions that we used for temperature and energetic analyses, especially changes in 1946 and 1977, were consistent with regime shifts identified in other studies. During the last 100 years, the most well-defined shifts in climate conditions in the North Pacific Ocean and western North America occurred in about 1925, 1947, and 1977 (Ware 1995; Beamish et al. 1999*b*). Length of day, the Aleutian low pressure index, land and sea surface temperatures, and chlorophyll *a* are some of the correlates used to identify the timing of these climate shifts (Beamish et al. 1999*b*).

Although major climate shifts were apparent in all indices, the three climate indices that we compared differed in several ways. The PNI and the CBI suggested that weaker climate shifts might have occurred in the coastal and inland areas about 1958 and 1969, while such changes were not as apparent in the PDO index. The strongest differences among the indices occurred from about 1977 to 1999. The positive CBI during spring and summer from 1981 to 1986 was probably the result of the strong El Niño event of 1982–1983 when water flows were greatly above normal (Piechota et al. 1997). Finally, the index of stream flows (CBI) shifted from below-normal flows for about 1985–1992 to above-normal flows after 1993. This change in the CBI in the early 1990s may be an indication of one of the stronger, long-term shifts in the climate such as the 1977 shift (Beamish et al. 1999*a*). Future studies might further investigate differences in oceanic and stream flow indices and test for correlations between stream flow indices and measures of adult salmon return to the basin.

Changing water temperature may affect salmonid mortality by directly influencing the feeding rate of piscivores and indirectly by affecting the growth rate of predators. Increased temperature during warm periods would cause faster growth of predators, larger size-at-age, and higher juvenile salmonid mortality, since larger predators consume disproportionately more salmonids than smaller predators (Vigg et al. 1991).

Demonstrating such indirect effects of temperature change on growth or other processes that are susceptible to many factors would be difficult, although the mechanisms that we have suggested appear quite plausible.

Our modeling required several assumptions about prey availability, diet, and predator movement. Our assumption of either fixed growth or fixed food availability had relatively little influence on the quantitative or qualitative outcome of the simulations that we ran. We also assumed a constant diet for predators, although diet has probably changed since the 1930s. For example, American shad (*Alosa sapidissima*) became established in the Columbia River in 1895 and their numbers increased dramatically in the lower Columbia River starting about 1958 (Quinn and Adams 1996). Since 1990, 2–3 million adult American shad have passed Bonneville Dam annually to spawn in the Columbia and Snake rivers. Juvenile American shad occur in the diet of northern squawfish and may supplement the growth of predators during late summer and fall (Petersen et al. 1994; J.H. Petersen, unpublished data). Other changes in predator diet could be related to impoundment following construction of hydroelectric dams or to changes in populations of piscivores (Poe et al. 1994). Finally, long-distance movements by predators could expose them to different temperature regimes. Radiotelemetry studies with northern squawfish and smallmouth bass suggest that individuals may migrate considerable distances to spawn, but they generally return and remain in the same general area after these reproductive movements (Martinelli and Shively 1997; J.H. Petersen, unpublished data).

No long-term population data are available for northern squawfish or other predators in the Columbia River, so we used a single hypothetical individual in our simulations. Thus, our estimates of the effects of temperature and climate change on predation were made on a per capita basis and do not reflect any changes in the size of predator populations that may have occurred since the 1930s. Impoundment of the river system has increased the area of certain habitats, perhaps leading to increased or decreased predator populations. The density of northern squawfish is high in tailraces and forebays of dams (Ward et al. 1995), so dam construction since 1933 might have increased the total available habitat and the predator population. On the other hand, fairly high densities of northern squawfish were recently observed in free-flowing reaches of the Columbia River (Hanford Reach) and the lower Snake River (J.H. Petersen, unpublished data), suggesting that impoundment may not have greatly changed the total number of northern squawfish in the system. Predaceous smallmouth bass, walleye, and channel catfish were introduced into the river system prior to 1900, and their numbers likely increased concurrent with impoundment of the river, although we have no long-term population estimates to confirm this (Poe et al. 1994).

Climate changes suggested by the three indices coincided with major dam building efforts on the mainstem Columbia and Snake rivers beginning in 1933, making it difficult to know exactly how much of the predicted predation differences among periods was due to climate shifts rather than impoundment of the system. Most of the dams on the Columbia and Snake rivers are "run-of-the-river" dams with little storage capacity and flushing rates of <4 days. Jaske and Goebel (1967) and Jaske and Synoground (1970) concluded that the erection of low-head dams on the Columbia River (prior to 1970) produced only a minimal increase in the average temperature of the river. Average June–July temperatures in the Columbia River during 1954–1990 were significantly correlated with temperatures in the Fraser River in British Columbia ($P < 0.01$; Quinn and Adams 1996). Since the Fraser River has not had extensive hydroelectric development, this correlation suggests regional temperature control through snowmelt, tributary warming, precipitation, and other large-scale climate-related processes. The most significant temperature effect of impoundment in the system may be extension of the warmwater period: earlier warming and later cooling within a year (Quinn and Adams 1996; Quinn et al. 1997). At Bonneville Dam on the lower Columbia River, for example, 15.5°C water occurs ~30 days earlier in the spring and remains ~17 days later in the fall, based on data from 1950 to 1993 (Quinn and Adams 1996). Earlier warming and later cooling of water within a year in the impounded Columbia River is accounted for in the model estimates of predation rate, since we used average monthly temperatures per climate period.

The annual hydrograph has obviously been modified by construction of dams, with lower spring and summer flows after impoundment (Ebel et al. 1989), but flow still varies considerably from year to year and presumably modulates water temperature. For example, average June flow varied from 3511 to 13 620 $m^3 \cdot s^{-1}$ on the Columbia River at The Dalles Dam for 1973–1994. It is difficult to correlate year-to-year temperature changes with the timing of dam construction, especially since there are both increases and decreases in these temperatures over a few years. The most dramatic change in the Columbia River occurred between 1955 and 1958 when the average daily temperature increased over 4°C. Only two dams, Chief Joseph (1955, flushing rate 2.6 days) and The Dalles (1957, flushing rate 1.1 days), came into service on the Columbia River during this 4-year period (Ebel et al. 1989). Beginning about 1975, summer water temperatures throughout the Columbia River have risen fairly steadily (Quinn et al. 1997), suggesting broad-scale climate effects, since all dams were operational by the early 1970s, except Revelstoke Dam in British Columbia, completed in 1984. Additional analyses of dam and climate effects on water temperatures in the Columbia River Basin would be helpful, perhaps using temperature models driven by local weather conditions.

We limited our analyses to climate regime shifts during the last 70 years, but future trends in global temperature or short-term events could increase predation loss in the Columbia River. Hamlet and Lettenmaier (1999) used two global climate models and a Columbia River hydrology model to predict changes in flow over the next 100 years. By 2045, for example, they predicted a reduction in summer flow at The Dalles Dam of 11–25% and an increase in summer air temperature of 1–3°C, the variation depending on which global model was used. Such flow and air temperature changes might translate into significant changes in water temperature and predation losses. Superimposed on this gradual warming trend are the shorter scale fluctuations of climatic regimes and El Niño events (Hamlet and Lettenmaier 1999). Co-occurrence of a warm climate regime and low flows from an El Niño event, combined with gradual global warming, might produce especially high water temperatures for a period and high predation losses for juvenile salmonids.

In conclusion, we demonstrated how an important source of mortality for juvenile salmonids, freshwater predation, could be influenced by water temperature changes driven by climatic regime shifts. Predation may also be important to salmonids in estuarine and nearshore ocean environments (Pearcy 1992), further emphasizing the need to understand climate phenomena at large spatial scales. Models that link the ocean, atmosphere, hydrology, and temperature are becoming available (e.g., Hamlet and Lettenmaier 1999) and perhaps can be combined with mechanistic models of predation and salmon movements (e.g., Zabel et al. 1998; Petersen and DeAngelis 2000) to make useful predictions about mortality and population recovery.

Acknowledgements

Much of this work was supported by contracts with the Bonneville Power Administration, administered by Bill Maslen, which we appreciate. Preparation of the manuscript also benefited from support to J.F.K. from the University of Wisconsin Graduate School and the National Center for Ecological Analysis and Synthesis, University of California at Santa Barbara. Tom Quinn stimulated our original thinking about the effects of temperature change in the Columbia River. Dena Gadomski, Barry Smith, and an anonymous reviewer made many helpful comments on earlier drafts of the manuscript.

References

Bartell, S.M., Breck, J.E., Gardner, R.H., and Brenkert, A.L. 1986. Individual parameter perturbation and error analysis of fish bioenergetics models. Can. J. Fish. Aquat. Sci. **43**: 160–168.

Beacham, T.D., and Murray, C.B. 1990. Temperature, egg size, and development of embryos and alevins of five species of Pacific salmon: a comparative analysis. Trans. Am. Fish. Soc. **119**: 927–945.

Beamesderfer, R.C., Ward, D.L., and Nigro, A.A. 1996. Evaluation of the biological basis for a predator control program on northern squawfish (*Ptychocheilus oregonensis*) in the Columbia and Snake rivers. Can. J. Fish. Aquat. Sci. **53**: 2898–2908.

Beamish, R.J., McFarlane, G.A., and Thomson, R.E. 1999*a*. Recent declines in the recreational catch of coho salmon (*Oncorhynchus kisutch*) in the Strait of Georgia are related to climate. Can. J. Fish. Aquat. Sci. **56**: 506–515.

Beamish, R.J., Noakes, D.J., McFarlane, G.A., Klyashtorin, L., Ivanov, V.V., and Kurashov, V. 1999*b*. The regime concept and natural trends in the production of Pacific salmon. Can. J. Fish. Aquat. Sci. **56**: 516–526.

Bradford, M.J. 1995. Comparative review of Pacific salmon survival rates. Can. J. Fish. Aquat. Sci. **52**: 1327–1338.

Cayan, D.R., and Peterson, D.H. 1989. The influence of North Pacific atmospheric circulation on streamflow in the west. Geophys. Monogr. **55**: 375–397.

Cayan, D.R., Dettinger, M.D., Diaz, H.F., and Graham, N.E. 1998. Decadal variability of precipitation over western North America. J. Clim. **11**: 3148–3166.

Cayan, D.R., Redmond, K.T., and Riddle, L.G. 1999. ENSO and hydrologic extremes in the western United States. J. Clim. **12**: 2881–2893.

Ebbesmeyer, C.C., and Strickland, R.M. 1995. Oyster condition and climate: evidence from Willapa Bay. Publication WSG-MR 95-02, Washington Sea Grant Program, University of Washington, Seattle, Wash.

Ebel, W.J., Becker, C.D., Mullan, J.W., and Raymond, H.L. 1989. The Columbia River — toward a holistic understanding. *In* Proceedings of the International Large River Symposium. *Edited by* D.P. Dodge. Can. Spec. Publ. Fish. Aquat. Sci. No. 106. pp. 205–219.

Elliott, J.M., Hurley, M.A., and Maberly, S.C. 2000. The emergence period of sea trout fry in a Lake District stream correlates with the North Atlantic Oscillation. J. Fish Biol. **56**: 208–210.

Emlen, J.M. 1995. Population viability of the Snake River chinook salmon (*Oncorhynchus tshawytscha*). Can. J. Fish. Aquat. Sci. **52**: 1442–1448.

Francis, R.C., and Hare, S.R. 1994. Decadal-scale regime shifts in the large marine ecosystems of the Northeast pacific: a case for historical science. Fish. Oceanogr. **3**: 279–291.

Hamlet, A.F., and Lettenmaier, D.P. 1999. Effects of climate change on hydrology and water resources in the Columbia River Basin. J. Am. Water Resour. Assoc. **35**: 1597–1623.

Hare, S.R., Mantua, N.J., and Francis, R.C. 1999. Inverse production regimes: Alaska and west coast Pacific salmon. Fisheries (Bethesda), **24**: 6–14.

Jaske, R.T., and Goebel, J.B. 1967. Effects of dam construction on temperatures of Columbia River. J. Am. Waterworks Assoc. **59**: 935–942.

Jaske, R.T., and Synoground, M.O. 1970. Effect of Hanford plant operations on the temperature of the Columbia River. AEC Research and Development Report (BNWL-1345, UC-70). Atomic Energy Commission, Richland, Wash.

Kitchell, J.F., Stewart, D.J., and Weininger, D. 1977. Applications of a bioenergetics model to yellow perch (*Perca flavescens*) and walleye (*Stizostedion vitreum vitreum*). J. Fish. Res. Board Can. **34**: 1922–1935.

Lins, H.F., and Slack, J.R. 1999. Streamflow trends in the United States. Geophys. Res. Lett. **26**: 227–230.

Mantua, N.J., Hare, S.R., Zhang, Y., Wallace, J.M., and Francis, R.C. 1997. A Pacific interdecadal climate oscillation with impacts on salmon production. Bull. Am. Meteorol. Soc. **78**: 1069–1079.

Martinelli, T.L., and Shively, R.S. 1997. Seasonal distribution, movements, and habitat associations of northern squawfish in two lower Columbia River reservoirs. Regul. Rivers Res. Manage. **13**: 543–556.

National Marine Fisheries Service. 2000. A standardized quantitative analysis of risks faced by salmonids in the Columbia River Basin. Report of the Cumulative Risk Initiative, Northwest Fisheries Science Center, Seattle, Wash.

National Research Council. 1996. Upstream: salmon and society in the Pacific Northwest. National Academy Press, Washington, D.C.

Nehlsen, W., Williams, J.E., and Lichatowich, J.A. 1991. Pacific salmon at the crossroads: stocks at risk from California, Oregon, Idaho, and Washington. Fisheries (Bethesda), **16**: 4–21.

Pearcy, W.G. 1992. Ocean ecology of North Pacific salmonids. Washington Sea Grant Program, University of Washington, Seattle, Wash.

Petersen, J.H. 1994. Importance of spatial pattern in estimating predation on juvenile salmonids in the Columbia River. Trans. Am. Fish. Soc. **123**: 924–930.

Petersen, J.H., and DeAngelis, D.L. 2000. Dynamics of prey moving through a predator field: a model of migrating juvenile salmon. Math. Biosci. **165**: 97–114.

Petersen, J.H., and Ward, D.L. 1999. Development and corroboration of a bioenergetics model for northern pikeminnow feeding on juvenile salmonids in the Columbia River. Trans. Am. Fish. Soc. **128**: 784–801.

Petersen, J.H., Gadomski, D.M., and Poe, T.P. 1994. Differential predation by northern squawfish (*Ptychocheilus oregonensis*) on live and dead juvenile salmonids in the Bonneville Dam tailrace (Columbia River). Can. J. Fish. Aquat. Sci. **51**: 1197–1204.

Piechota, T.C., Dracup, J.A., and Fovell, R.G. 1997. Western U. S. streamflow and atmospheric circulation patterns during El Nino – Southern Oscillation. J. Hydrol. **201**: 249–271.

Poe, T.P., Hansel, H.C., Vigg, S., Palmer, D.E., and Prendergast, L.A. 1991. Feeding of predaceous fishes on out-migrating juvenile salmonids in John Day Reservoir, Columbia River. Trans. Am. Fish. Soc. **120**: 405–420.

Poe, T.P., Shively, R.S., and Tabor, R.A. 1994. Ecological consequences of introduced piscivorous fishes in the lower Columbia and Snake rivers. *In* Theory and application in fish feeding ecology. *Edited by* D.J. Stouder, K.L. Fresh, and R.J. Feller. University of South Carolina Press, Columbia, S.C. pp. 347–360.

Quinn, T.P., and Adams, D.J. 1996. Environmental changes affecting the migratory timing of American shad and sockeye salmon. Ecology, **77**: 1151–1162.

Quinn, T.P., Hodgson, S., and Peven, C. 1997. Temperature, flow, and the migration of adult sockeye salmon (*Oncorhynchus nerka*) in the Columbia River. Can. J. Fish. Aquat. Sci. **54**: 1349–1360.

Rieman, B.E., Beamesderfer, R.C., Vigg, S., and Poe, T.P. 1991. Estimated loss of juvenile salmonids to predation by northern squawfish, walleyes and smallmouth bass in John Day Reservoir, Columbia River. Trans. Am. Fish. Soc. **120**: 448–458.

Roell, M.J., and Orth, D.J. 1993. Trophic basis of production in stream-dwelling smallmouth bass, rock bass, and flathead catfish in relation to invertebrate bait harvest. Trans. Am. Fish. Soc. **122**: 46–62.

Shuter, B.J., and Post, J.R. 1990. Climate, population variability, and the zoogeography of temperate fishes. Trans. Am. Fish. Soc. **119**: 314–336.

Slack, J.R., and Landwehr, J.M. 1992. Hydro-Climatic Data Network (HCDN) streamflow data set, 1874–1988. U.S. Geol. Surv. Water Resour. Invest. Rep. 93-4076.

Smith, B.D. 2000. Trends in wild adult steelhead (*Oncorhynchus mykiss*) abundance for snowmelt-driven watersheds of British Columbia in relation to freshwater discharge. Can. J. Fish. Aquat. Sci. **57**: 285–297.

Vigg, S., Poe, T.P., Prendergast, L.A., and Hansel, H.C. 1991. Rates of consumption of juvenile salmonids and alternative prey fish by northern squawfish, walleyes, smallmouth bass, and channel catfish in John Day Reservoir, Columbia River. Trans. Am. Fish. Soc. **120**: 421–438.

Walters, C.J., and Ward, B.R. 1998. Is solar UV radiation responsible for declines in marine survival rates of anadromous salmonids that rear in small streams? Can. J. Fish. Aquat. Sci. **55**: 2533–2538.

Ward, D.L., Petersen, J.H., and Loch, J.J. 1995. Index of predation on juvenile salmonids by northern squawfish in the lower and middle Columbia River and in the lower Snake River. Trans. Am. Fish. Soc. **124**: 321–334.

Ware, D.M. 1995. A century and a half of change in the climate of the NE Pacific. Fish. Oceanogr. **4**: 267–277.

Zabel, R.W., Anderson, J.J., and Shaw, P.A. 1998. A multiple-reach model describing the migratory behavior of Snake River yearling chinook salmon (*Oncorhynchus tshawytscha*). Can. J. Fish. Aquat. Sci. **55**: 658–667.

Within-drainage population genetic structure of the freshwater fish *Pseudomugil signifer* (Pseudomugilidae) in northern Australia

D.J. McGlashan, J.M. Hughes, and S.E. Bunn

Abstract: Dendritic channel patterns have the potential to isolate populations within drainages, depending on the relative position within the stream hierarchy of the populations. We investigated the extent of genetic subdivision in the Australian freshwater fish *Pseudomugil signifer* (Kner) (Pseudomugilidae) from two drainages in northern Queensland, Australia, using allozyme techniques. The drainages were adjacent and had similar channel patterns each with two major subcatchments coalesced to an estuarine confluence. Analysis of 30 sites across the two drainages revealed that although there was significant genetic variation among sites in both drainages, this was not between the two subcatchments in either case. This result did not support predictions of the stream hierarchy model (SHM), which would predict higher levels of variation among subcatchments than within them, nor did it suggest that estuarine conditions represent a significant barrier to dispersal in this species. More variation was among sites within each subcatchment. Multidimensional scaling plots revealed that, although most sites within a drainage were similar to one another, outlier sites occurred in each drainage, so correlations between genetic distance and geographic distance were weak. We suggest that the distance between sites and the probability of connectivity between sites may better explain the observed distribution of genetic diversity.

Résumé : Selon leur position relative dans la hiérarchie d'un cours d'eau, les populations peuvent potentiellement être isolées dans le bassin versant par la structure dendritique des chenaux. À l'aide de techniques allozymatiques, nous avons étudié l'étendue de la subdivision génétique chez le poisson d'eau douce australien *Pseudomugil signifer* (Kner) (Pseudomugilidae) dans deux bassins versants du nord du Queensland en Australie. Les bassins sont adjacents et possèdent des arrangements de chenaux similaires; chacun comporte deux sous-bassins majeurs qui se fusionnent en une confluence estuarienne. L'analyse de 30 sites répartis dans les deux bassins indique que, bien qu'il y ait une variation génétique significative d'un site à l'autre dans les deux bassins, la variation ne s'établit dans aucun des deux cas entre les deux sous-bassins. Ce résultat ne s'accorde pas avec les prédictions du modèle hiérarchique des cours d'eau (SHM) qui prédit l'existence de variations plus grandes d'un sous-bassin à un autre qu'à l'intérieur d'un même sous-bassin; il ne laisse pas non plus croire que les conditions estuariennes forment une barrière sérieuse à la dispersion chez cette espèce. Il y a plus de variation entre les sites à l'intérieur de chaque sous-bassin. Des cadrages multidimensionnels révèlent que, bien que la plupart des sites d'un même bassin soient semblables, il y a des sites aberrants dans chaque bassin et donc les corrélations entre les distances génétique et géographique sont faibles. La distance entre les sites et la probabilité de connectivité entre les sites peuvent, selon nous, mieux expliquer la distribution de la diversité génétique que nous avons observée.

[Traduit par la Rédaction]

Introduction

There are many studies that demonstrate that genetic differentiation is greatest among populations of freshwater organisms that inhabit streams in different drainages (see Ward et al. 1994). However, there are fewer studies that have explored the levels of genetic differentiation among populations within drainages to assess the influence of stream structure, distance, and historical processes on population structure (Lu et al. 1997).

The structure of drainage networks isolates populations that inhabit different drainages but also partitions populations within a drainage. Meffe and Vrijenhoek (1988) proposed the stream hierarchy model (SHM) to account for the influence of the hierarchical nature of streams on the distribution of genetic diversity. If the isolation of populations is a function of their relative position in the drainage that they inhabit, then at equilibrium, one would expect differentiation to be greatest among drainages, decreasing to the least differentiation among populations within streams.

The probability of connectedness between two populations within a drainage is also likely to be influenced by other extrinsic factors such as the unidirectional flow of streams and distance. In studies of the Trinidadian guppy, reduced levels of

Received November 30, 2000. Accepted June 8, 2001. Published on the NRC Research Press Web site at http://cjfas.nrc.ca on August 30, 2001.
J16116

D.J. McGlashan, J.M. Hughes,[1] and S.E. Bunn. Co-operative Research Centre for Tropical Rainforest Ecology and Management, Australian School of Environmental Studies, Griffith University, Nathan, Queensland, Australia, 4111.

[1] Corresponding author (e-mail: jane.hughes@mailbox.gu.edu.au).

DOI: 10.1139/cjfas-58-9-1842

polymorphism in headwater streams were attributed to founder effects, while downstream sites were influenced by isolation by distance and physical barriers (Shaw et al. 1994). A bias towards downstream over upstream gene flow in stream systems has been suggested to result in fewer alleles, greater differentiation, and lower heterozygosity within upstream populations relative to within downstream populations (Hernandez-Martich and Smith 1997). Slatkin (1993) showed that at genetic drift – gene flow equilibrium, a log linear relationship between geographic distance and genetic differentiation exists, assuming a one- or two-dimensional stepping stone model of gene flow (also see Hutchison and Templeton 1999).

Finally, intrinsic demographic dispersal and extinction/colonisation processes at the localised within-drainage scale may be important in levels of genetic differentiation among populations. Habitat size, and by inference population size, presumably diminishes with distance upstream. Smaller upstream populations may be more prone to extinction and recolonisation events. Whitlock and McCauley (1990) revealed that these metapopulation processes could increase genetic differentiation among populations. Also, as drainage patterns change through geological time, patterns of connectivity may be altered. There may be a shift in the drift – gene flow equilibrium between populations depending on the frequency of the demographic or environmental perturbation and the time elapsed.

We investigated the levels of genetic differentiation among populations within drainages of a freshwater fish, the Pacific blue eye *Pseudomugil signifer* (Kner) (Pseudomugilidae). Our first aim was to partition the distribution of genetic diversity within drainages to test the SHM. As a corollary, we also addressed whether estuarine confluences isolate populations from different subcatchments. Second, we examined the relationship between geographic distance and genetic differentiation among populations within drainages as an alternative to the SHM. Finally, we attempted to construct a conceptual model of within-drainage population structure that integrated the distribution of genetic diversity.

Materials and methods

Study species

Pseudomugil signifer is a small, abundant fish that is continuously distributed from Cooktown, Queensland, to Narooma, New South Wales, along the eastern coast of Australia (Allen 1989) as well as the western coast of Cape York Peninsula (Saeed et al. 1989) (Fig. 1). Semple (1986, 1991) suggested that spawning occurred year-round with approximately 100 eggs produced. Eggs are demersal and adhesive and range from 1.1 to 1.8 mm in diameter, and larvae are 4–5 mm at hatching. Age at maturity is approximately 6 months, and longevity is 1 year. *Pseudomugil signifer* can tolerate brackish to fully marine conditions, being found in mangrove areas (e.g., Blaber 1980) and several offshore islands such as Lizard Island (Saeed et al. 1989) and in mangrove habitat on the Low Isles (Hadfield et al. 1979). Accordingly, this species appears to have a moderate dispersal potential.

Sampling strategy

The Mulgrave-Russell and Johnstone rivers were selected to assess patterns of variation among populations within drainages (Fig. 1). They share similar dendritic channel patterns, in that two major subcatchments coalesce at an estuarine confluence and then empty to the ocean. We collected tributary and main-channel samples from throughout the lowland sections of the two drainages. In addition, we were interested in the amount of variation within a reach of stream, so we collected two samples within Behana Creek (BHA and BHB), which were 3 km distant, and two samples from the Little Mulgrave River and a tributary (LMA and LMB), which were approximately 500 m apart (Fig. 1). Replicates from three sites (BHB, HYC, and SJR) were collected in 1996 and 1997 to examine temporal changes in allele frequency over approximately two generations. Sample site information is given in Fig. 1 and sample sizes are given in Appendix Tables A1 and A2.

Laboratory procedures

Samples were prepared for allozyme electrophoresis and 18 enzyme systems screened following the procedures of McGlashan and Hughes (2000) based on protocols in Richardson et al. (1986). After screening, six loci encoding five enzyme systems were reliably polymorphic. The enzymes used were glucose-6-phosphate isomerase (EC 5.3.1.9; *GPI-1** and *GPI-2** loci), phosphoglucomutase (EC 5.4.2.2; *PGM** locus), alcohol dehydrogenase (EC 1.1.1.1, *ADH** locus), tripeptide aminopeptidase (EC 3.4.11.4; *PEPB** locus), and phosphogluconate dehydrogenase (EC 1.1.1.44; *PGDH** locus). Allozymes were used as the marker of choice, as six polymophic loci had already been identified from a broad geographic study. These loci, although they varied in their levels of variability, all showed highly significant F_{ST} values on the broad scale ranging from 0.193 to 0.814 (D.J. McGlashan and J.M. Hughes, unpublished data).

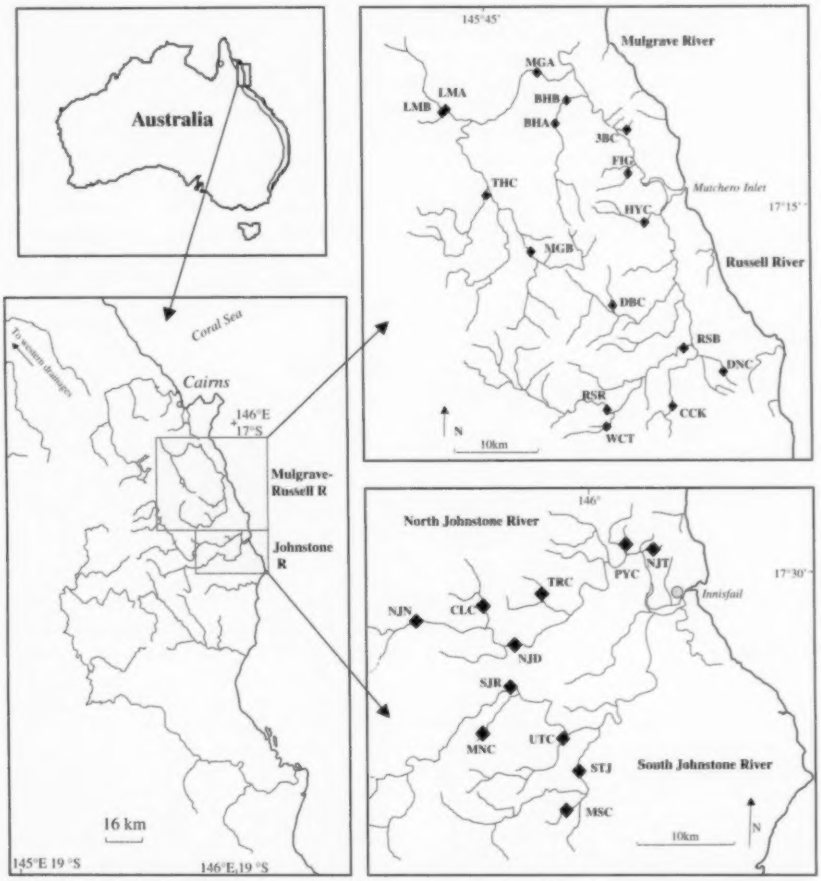
Statistical analysis

The probability test of Guo and Thompson (1992) was used to test conformity with Hardy–Weinberg proportions of populations using GENEPOP (Raymond and Rousset 1995) by testing for both the alternative hypotheses of heterozygote deficiency or excess. Exact *P* tests for detecting linkage disequilibrium were used to test the independence of allozyme loci, as performed by GENEPOP. Exact tests of population differentiation were used to assess the heterogeneity between geographically near sites (i.e., BHA and BHB96, and LMA and LMB), among all sites within a drainage, and between the temporal replicates. Within each subcatchment, sites were categorised as upstream or downstream sites and the mean heterozysity (*H*) calculated.

We used analysis of molecular variance techniques to partition the distribution of genetic diversity among populations (Schneider et al. 1997) using 10 000 permutations to derive *P* values. There were two levels to the hierarchical analysis: among subcatchments within drainages and among sites within a subcatchment. Each site was assigned to its respective subcatchment to form the hierarchies as detailed in notation below. For the Mulgrave-Russell drainage, sites were assigned to the Mulgrave (MGA, LMB, BHB96, 3BC, FIG, THC, and MGB) or Russell subcatchment (HYC96, DBC, RSB, DNC, RSR, CCK, and WCT). For the Johnstone drainage, sites were assigned to either the North Johnstone (PYC, NJT, CLC, NJN, TRC, NJD) or South Johnstone subcatchment (SJR96, MNC, UTC, STJ, MSC).

Qualitative patterns of population relationships were summarised by multidimensional scaling (MDS) of Rogers' (1972) genetic distance, a metric measure suitable for MDS. We used the methods of Slatkin (1993) to investigate the relationship between levels of genetic differentiation ($\hat{M}$) and geographic (stream) distance. To quantify $\hat{M}$, we used Nei's (1973) measure, G_{ST}, to avoid negative pairwise estimates. Stream distances were measured from 1 : 50 000 or 1 : 100 000 topographical maps using Summagraphics SummaSketch Plus software.

Fig. 1. Map illustrating sampling sites (◆) for *P. signifer* populations in the Mulgrave-Russell and Johnstone drainages. Site abbreviation (bold), site name, and subcatchment status: **MGA**, Mulgrave River A, Mulgrave; **LMB**, No Name Creek, Mulgrave; **LMA**, Little Mulgrave River A, Mulgrave; **BHA**, Behana Creek A, Mulgrave; **BHB96**, Behana Creek B (1996 sample), Mulgrave; **BHB97**, Behana Creek B (1997 sample), Mulgrave; **3BC**, 3rd Bridge Creek, Mulgrave; **FIG**, Figtree Creek, Mulgrave; **THC**, Toohey Creek, Mulgrave; **MGB**, Mulgrave River B, Mulgrave; **HYC96**, Harvey Creek (1996 sample), Russell; **HYC97**, Harvey Creek (1997 sample), Russell; **DBC**, Double Barrell Creek, Russell; **RSB**, Russell River B, Russell; **DNC**, Dinner Creek, Russell; **RSR**, Russell River, Russell; **CCK**, Canal Creek, Russell; **WCT**, Woopen Creek Tributary, Russell; **PYC**, Polly Creek, North Johnstone; **NJT**, North Johnstone Tibutary, North Johnstone; **CLC**, Culla Creek, North Johnstone; **NJN**, North Johnstone River at Nerada, North Johnstone; **TRC**, Tregonthanaan Creek, North Johnstone; **NJD**, North Johnstone River at Dickah Hill, North Johnstone; **SJR96**, South Johnstone River (1996 sample), South Johnstone; **SJR97**, South Johnstone River (1997 sample), South Johnstone; **MNC**, Meingan Creek, South Johnstone; **UTC**, Utchee Creek, South Johnstone; **STJ**, Stewart Creek SJ, South Johnstone; **MSC**, Miskin Creek, South Johnstone. Sites LMA and LMB were pooled to form LMR and BHA and BHB96 were pooled to form BHC after exact tests of population differentiation revealed no significant differences between these sites. Sample sizes are listed in Appendices 1 and 2. Shading in top left map represents the approximate distribution of *P. signifer*.


Australia
Coral Sea
Cairns
To western drainages
146°E
17°S
Mulgrave-Russell R
Johnstone R
16 km
145°E 19 °S
146°E,19 °S
145°45'
Mulgrave River
MGA
LMA
LMB
BHB
BHA
3BC
FIG
Mutchero Inlet
THC
HYC
17°15'
MGB
Russell River
DBC
RSB
DNC
RSR
CCK
WCT
N
10km
146°
North Johnstone River
PYC
NJT
17°30'
Innisfail
TRC
NJN
CLC
NJD
SJR
MNC
UTC
STJ
South Johnstone River
MSC
10km
N

Results

Variation within populations

In total, 30 sites were sampled from the Mulgrave-Russell and Johnstone drainages. Two sites showed significant heterozygote deficient multilocus departures from Hardy–Weinberg expectations after adjusting the level of significance for multiple tests using the sequential Bonferroni method. The sites were Culla Creek in the North Johnstone subcatchment (CLC, $P = 0.0001$) and Woopen Creek tributary in the Russell subcatchment (WCT, $P = 0.0003$). Tests for linkage disequilibrium between loci revealed no significant results after the sequential Bonferroni adjustment of significance levels.

Population differentiation

At the smallest spatial scale, there was no evidence of significant heterogeneity at any of the six allozyme loci using exact tests of differentiation (Raymond and Rousset 1995) for LMA/LMB (overall $P = 0.75$) or BHA/BHB (overall $P = 0.65$). Data were therefore combined from sites LMA and LMB (forming site LMR) and BHA and BHB96 (forming site BHC) in all subsequent analyses. After applying the

Bonferroni adjustment, there were no significant differences between temporal replicates of site BHB (overall loci P = 0.13), HYC (P = 0.31), or SJR (P = 0.41) as determined by exact tests. They were also combined.

Overall jackknifed estimates of F_{ST} among populations within each of the Mulgrave-Russell (F_{ST} = 0.19 ± 0.10 P < 0.0001) and Johnstone drainages (F_{ST} = 0.28 ± 0.06, P < 0.0001) showed that there were significant levels of population subdivision. Exact tests of pairwise population differentiation averaged over loci revealed many significant results. After applying the sequential Bonferroni adjustment, 45 of 91 Mulgrave-Russell comparisons were significant and in the Johnstone, 50 of 55. Perusal of gene frequencies among populations of the two drainages further exemplifies the high degree of population differentiation. For instance, in the Mulgrave-Russell drainage, there was a high frequency of the *ADH*3* allele in populations in Behana Creek (BHA, BHB96, and BHB97) relative to other Mulgrave River populations (Appendix 1). Among Johnstone River sites, the frequency of the *PEPB*2* allele at site CLC (North Johnstone) was 1.0, while at NJN, little more than 8 km stream distance away, the frequency was 0.24 (Appendix 2). For these same two sites, the frequency of the *GPI-1*3* allele was 1.0 at CLC and 0.16 at NJN. At the *PEPB** locus, site MNC was fixed for a different allele than that found at CLC, sites that are within the same drainage (Appendix 2). At MSC (South Johnstone), the frequency of the *PEPB*3* allele was 0.57, while at STJ 5 km downstream, it was 0.84 (Appendix 2). Some sites also showed very low levels of heterozygosity at all loci (e.g., MNC) and tended to be upstream or tributary sites (Appendix 2), with the mean heterozygosity lower upstream than downstream for three of the four subcatchments (Table 1).

MDS ordination of Rogers' (1972) genetic distance revealed no clear separation by subcatchments and the presence of "outlier" sites which were divergent from their nearby counterparts (Fig. 2). For example, in the Mulgrave-Russell drainage, the position of site BHC in ordination space was widely divergent from other Mulgrave River populations (Fig. 2*a*), due mainly to the high frequency of the *ADH*3* allele (see Appendix 1). In the Johnstone drainage, site CLC, which had large gene frequency differences at the *ADH** and *PEPB** loci, was an outlier site (Fig. 2*b*).

Hierarchical analysis of variation

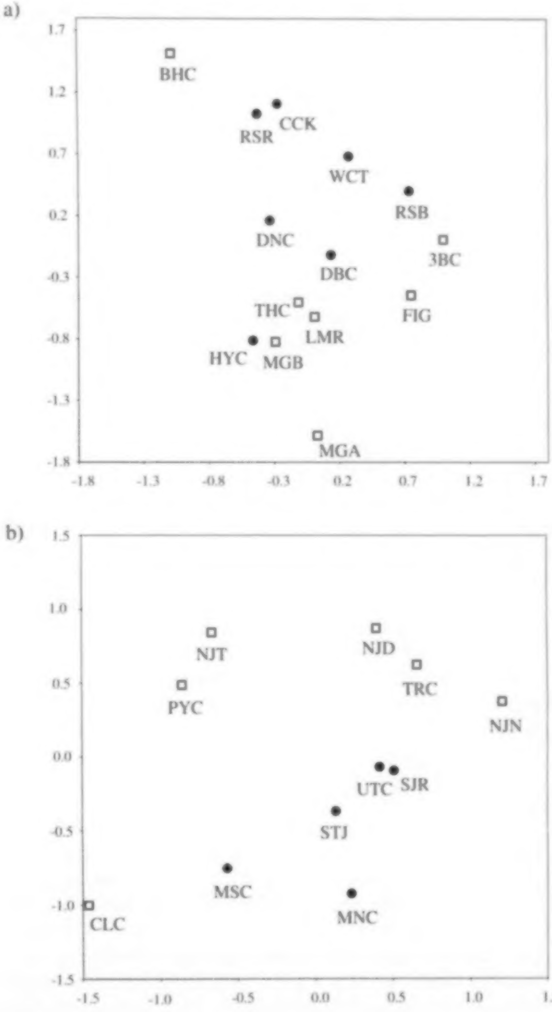
When the genetic variation was partitioned hierarchically, more variation among the Mulgrave-Russell sites was explained by the differences among sites within a subcatchment than between the subcatchments (15.1% compared with –2.1%) (Table 2). Indeed, the between-subcatchment component of variation was not significant (P = 0.91). Removing the outlier site (BHC) from the analysis resulted in only a small increase in the variation attributed between subcatchments within a drainage (data not shown).

The pattern among the Johnstone River sites was similar to that among the Mulgrave-Russell River sites. Genetic variation among sites within subcatchments (22.5%) was greater than between the subcatchments within the drainage (6.5%) (Table 2). Again, the between-subcatchment component was not significant (P = 0.08). When site CLC was removed from the analysis, there was only a small increase in the variation attributed between subcatchments relative to the drainage (data not shown).

Table 1. Mean heterozygosity (H) and standard error (in parentheses) for downstream and upstream sites in each subcatchment.

	Downstream	Upstream
Russell	0.0584 (0.0201)	0.0993 (0.0087)
Mulgrave	0.1003 (0.0115)	0.0339 (0.0076)
North Johnstone	0.2569 (0.0256)	0.1441 (0.0690)
South Johnstone	0.1420 (0.0247)	0.0903 (0.0492)

Fig. 2. MDS plots of Rogers' (1972) genetic distance for six allozyme loci of populations of *P. signifer* in the (*a*) Mulgrave-Russell (□, Mulgrave; ●, Russell; stress = 0.11) and (*b*) Johnstone drainages (□, North Johnstone; ●, South Johnstone; stress = 0.10). Site abbreviations as in Fig. 1.



Isolation by distance

There was a significant relationship between log($\hat{M}$) and

Table 2. Analysis of molecular variance for populations of *P. signifer* in the Mulgrave-Russell and Johnstone drainages.

Source	% variation	*F* statistic	*P*
Mulgrave-Russell drainage			
Between subcatchments	−2.1	−0.021	0.91 ± 0.0023
Among sites within subcatchments	15.1	0.148	<0.0001
Among all sites	13.0	0.130	<0.0001
Johnstone drainage			
Between subcatchments	6.2	0.062	0.087 ± 0.003
Among sites within subcatchments	22.5	0.239	<0.0001
Among all sites	28.6	0.286	<0.0001

log(stream distance) among all sites of the Mulgrave-Russell drainage (Fig. 3*a*) and among all sites of the Johnstone drainage (Fig. 3*b*). However, the coefficients of determination were low (0.07 and 0.19, respectively). The regression slopes describing the relationship between log($\hat{M}$) and log(stream distance) for all populations of the Mulgrave-Russell were approximately –0.5 and for the Johnstone, –0.65.

The log of stream distance was subsequently plotted against log($\hat{M}$) for populations of the Mulgrave and Russell subcatchments separately. Correlations were insignificant between sites within each subcatchment (Figs. 3*c* and 3*e*). Inspection of the scatterplots among Mulgrave sites only (Fig. 3*c*) identified comparisons between populations that had pairwise values of log($\hat{M}$) near zero or less. These were identified as comparisons involving site BHC (reflected in the MDS ordination, Fig. 2*a*), which when removed from the analysis resulted in a significant relationship between distance and genetic differentiation among populations within the Mulgrave subcatchment ($P = 0.048$, $r^2 = 0.20$) (Fig. 3*c*).

Similar patterns in the isolation by distance analyses to the Mulgrave-Russell were evident among populations of the Johnstone River. Stream distance did not explain any of the differentiation among populations of the North or South Johnstone subcatchments when considered separately (Figs. 3*d* and 3*f*). Inspection of the North Johnstone scatterplot (Fig. 3*d*) revealed comparisons between populations that had pairwise log($\hat{M}$) values of less than –0.5. These were identified as involving site CLC (reflected in the MDS ordination, Fig. 2*b*), which when removed from the analysis revealed a strong correlation between log(distance) and log($\hat{M}$) ($P = 0.002$, $r^2 = 0.78$) (Fig. 3*d*).

Discussion

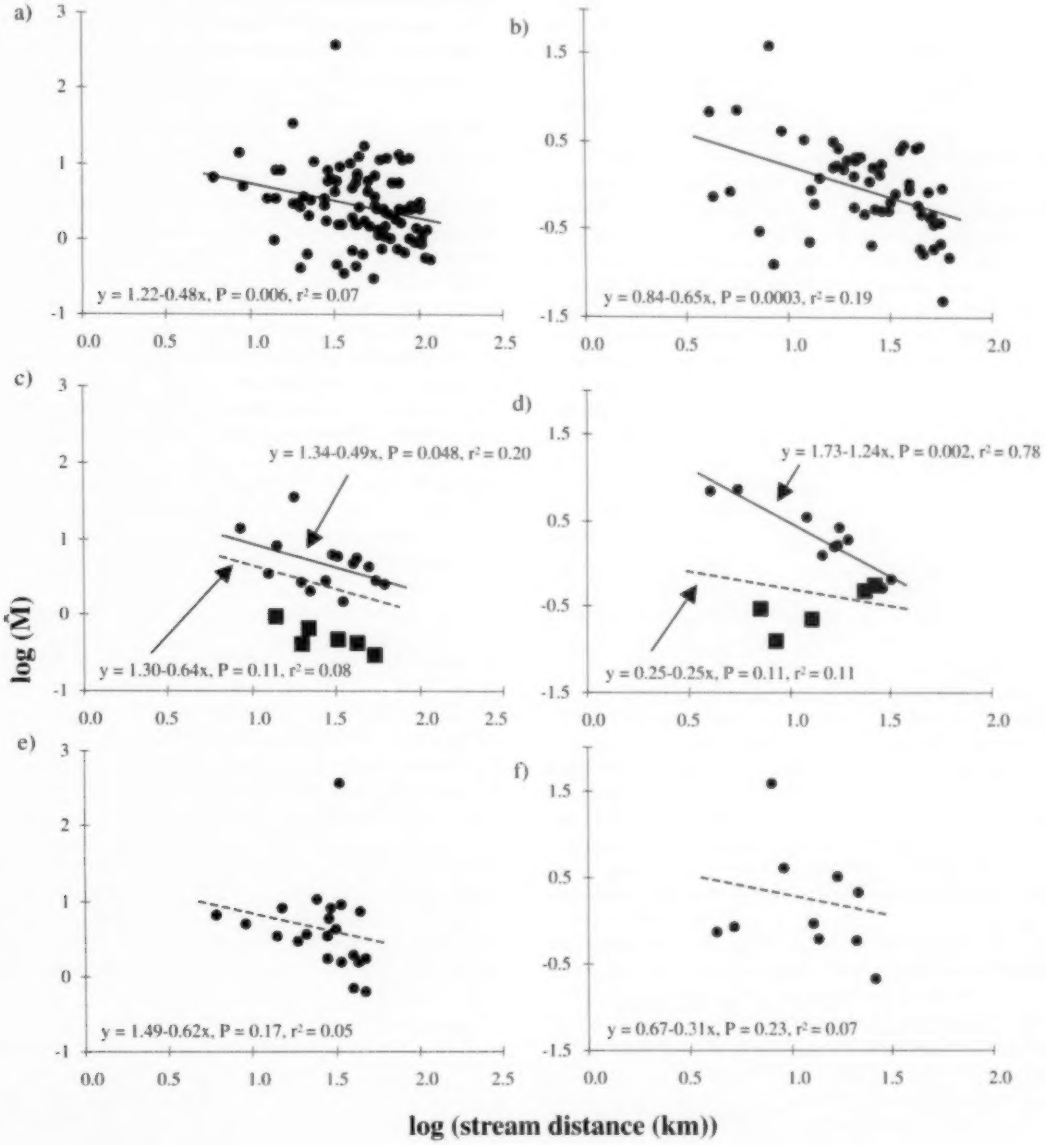
The present study makes the first thorough attempt at quantifying the within-drainage population structure of an Australian freshwater fish. In both of the drainages sampled, there were significant F_{ST} values, which indicated that populations were not panmictic within drainages. Studies in other geographic areas have also shown significant levels of population differentiation within drainages (e.g., Trinidadian guppies, Shaw et al. 1994; Florida freshwater fish, Baer 1998). Shaw et al. (1994) suggested that river order, isolation by distance, and barriers were important contributors to within-drainage gene frequency heterogeneity. A possible bias toward downstream over upstream gene flow may also be important (Shaw et al. 1994; Hernandez-Martich and Smith 1997). Below, we explore some of these issues in relation to the population structure of *P. signifer* within drainages.

Populations of *P. signifer* within drainages appear to have higher levels of connectivity than populations that inhabit separate drainages (42% of genetic variation was among drainages in a broad geographic study, McGlashan 2000), which is consistent with the SHM of Meffe and Vrijenhoek (1988). Thus, the isolation imposed by the drainage boundary appears to partition the distribution of genetic diversity observed. However, the SHM predicts that further partitioning of genetic diversity should also be dependent on divisions within drainages, which was not the case for *P. signifer*. Therefore, other factors must be important in determining the distribution of genetic diversity. The estuarine confluences that separate the Mulgrave from the Russell and the North Johnstone from the South Johnstone do not appear to be important determinants of population structure in this species. If they were, the partitioning of genetic variation among sites within systems should have been greater between subcatchments than among populations within subcatchments, which was not the case.

An alternative model for stream-dwelling species is isolation by distance (IBD). In this model, equilibrium between genetic drift and gene flow may be reached in species where the lifetime dispersal distance is less than the range, and a relationship between distance and genetic differentiation should be apparent (Wright 1943). In *P. signifer*, there was a significant association between genetic differentiation and distance, suggesting that an IBD model may be appropriate. The slopes of the relationships approximated –0.5, which Slatkin (1993) demonstrated is the theoretical expectation from the two-dimensional stepping stone model. This implies that for populations of *P. signifer*, the dendritic nature of streams may approximate a two-dimensional habitat more than a one-dimensional habitat. In other words, connectivity between populations is not necessarily decreased between populations separated by nodes in the stream hierarchy, compared with populations inhabiting the same linear stretch of stream.

The IBD model is, however, not entirely satisfactory for explaining population structure in *P. signifer* within drainages. Although significant, the relationship between geographic and genetic distance was weak; distance explained less than 20% of the genetic differentiation among populations inhabiting the Mulgrave-Russell and Johnstone drainages (Fig. 3). In addition, the relationship between genetic differentiation and geographic distance should be monotonic and the scattergram should have little spread in

Fig. 3. Scatterplots of log(stream distance) versus log($\hat{M}$) for populations of *P. signifer* in the (*a*) Mulgrave-Russell and (*b*) Johnstone drainages and subcatchments, (*c*) Mulgrave, (*d*) North Johnstone, (*e*) Russell, and (*f*) South Johnstone. The broken lines in Figs. 3*c*–3*f* indicate the line of best fit when all pairwise comparisons were considered. The solid line in Fig. 3*c* represents the line of best fit when comparisons involving site BHC (■) were removed from the comparison. The solid line in Fig. 3*d* represents the line of best fit when comparisons involving site CLC (■) were removed from the comparison.



values (i.e., high r^2) (Hutchison and Templeton 1999), which was not the case in the present study. Moreover, theoretical predictions suggest that nearer populations should approach equilibrium before more distant populations (see Slatkin 1993). When populations within subcatchments only were considered, there was little evidence for isolation by distance (noting that the power of the analysis was reduced through lower site numbers). Also, nearer sites often had a large spread of values in genetic differentiation – stream distance comparisons. It may be that there are simply high levels of recurrent gene flow between nearer sites (Hutchison and Templeton 1999). However, the large frequency differences between geographically close sites (e.g., CLC and NJN) may suggest that high levels of gene flow occur only between some nearby sites.

A better model than either the SHM or the IBD model of *P. signifer* population structure within drainages may require the consideration of metapopulation processes such as extinction/

colonisation events and other demographic parameters. Slatkin (1977) and Whitlock and McCauley (1990) suggested that the genetic consequences of founding events can be complex and depend on the number of founders and the characteristics of the founding population(s). In the current study, distinctive populations were recognised from MDS ordination of Rogers' (1972) genetic distance. For instance, site CLC was fixed at two loci that were variable just downstream (e.g., *PGI-1** and *PEPB** loci). Sites in Behana Creek in the Mulgrave River had high frequencies of the *ADH*3* allele that had a low frequency in other nearby sites. These outlier populations were in small, upstream habitats, which may be more prone to stochastic extinction/colonisation events. When these sites were removed from their respective analyses, the predictive power of distance increased dramatically, especially when site CLC was removed.

A more powerful model of gene flow may thus involve a mixed model that considers the distance between sites as well as the probability of connectivity and size of populations that inhabited various parts of a stream system. It may be that nearby populations are more likely to be connected than more distant populations (e.g., isolation by distance plots without outlier sites). However, populations in smaller headwater streams may be more prone to the stochastic effects of drift which may increase the variance in gene frequencies of headwater sites compared with other downstream populations (e.g., isolation by distance plots with outlier sites). If gene flow is reduced to the smaller, more isolated headwater streams, they will take longer to approach equilibrium than populations that have greater levels of gene flow because equilibrium approaches faster at higher migration rates (Whitlock 1992). Although this may be offset by the fact that larger populations (i.e., probably downstream) take longer to approach equilibrium than smaller populations (Whitlock 1992), the weak relationship between genetic differentiation and distance among sites in *P. signifer* may be exacerbated by the inclusion of the smaller, isolated headwater populations which are out of equilibrium with the other populations. Whitlock (1992) demonstrated that estimates of F_{ST} can vary between 0.1 and 0.5 over approximately 20 generations with random fluctuations of migration rate. Values of F_{ST} in the present study were encompassed by this range.

The temporal stability of gene frequencies of sites that were sampled in 1996 and 1997 (approximately two generations) does not necessarily conform to the predictions from metapopulation models because the effective population size of the populations compared is unknown. If they were small, the temporal stability in gene frequencies lends support to the notion that sites sampled are subdivided with low levels of gene flow (e.g., Spruell et al. 1999). However, if they were large, the inertia of larger populations may not necessarily be contrary to the metapopulation concept. The lack of independent data on population size and the small number of generations between sampling periods preclude firm conclusions in this regard. It may be useful to sample the same populations in the future to document temporal changes in gene frequencies.

It is also important to consider other factors that may be contributing to variation in allele frequencies among populations of *P. signifer* in the Mulgrave-Russell and Johnstone drainages. Genetic drift and gene flow are expected to affect all loci equally, whereas the effects of selection are locus specific (e.g., Slatkin 1987). The high F_{ST} estimate among populations in the Mulgrave-Russell drainage was largely generated by frequency differences at the *ADH** locus, in which the frequency of the *ADH*3* allele was high in the Behana Creek and Russell River populations. If selection is favouring the *ADH*3* allele in these populations, there would need to be a high selection coefficient to account for the frequency differences. Major differences in the selection regime between these areas are not obvious; however, selection cannot be discounted as a force in shaping allozyme allele frequencies.

Another possible source of variation in the observed allele frequencies is that there was a bias in the sampling of individuals from only one or a few related families. Recently, Hansen et al. (1997) showed that sampling only limited numbers of families of brown trout which exhibited nonrandom distributions led to biased estimates of allele frequencies. They found that many juveniles within the same reach of stream had an otherwise rare mtDNA haplotype, and microsatellite analysis showed that there were likely only three full-sib families represented (Hansen et al. 1997). Assumptions underlying the Hardy–Weinberg equilibrium are that there is random mating within a large population (Richardson et al. 1986). In the present study, it is possible that some of the small tributary streams are inhabited by only a small number of families, which may have led to the deviations from Hardy–Weinberg proportions at some sites. Little is known about *P. signifer* abundance in the sampled streams or about their spawning behaviour and life history. However, some local groups of fish are dominated by a single male that aggressively defends an area (Semple 1986; Howe 1987), which may lead to assortative associations at a localised scale. The sampling strategy was designed to avoid the problem of sampling families, in that a reach of stream was sampled and not just a small area; however, in some of the smaller upstream sites, it may be that there was a bias toward a few families. By chance, some of these families may be monomorphic at one or more loci, thus biasing allele frequencies compared with samples gathered from larger, randomly mating populations. Studies of stream insects have revealed deviations from Hardy–Weinberg proportions that have been attributed to a small number of females contributing to egg deposition in a reach of stream (Schmidt et al. 1995; Hughes et al. 1998).

The fact that three of the six allozyme loci used in the analysis showed no variation between many sites (i.e., were fixed for the same allele) may have weakened the power to detect a strong isolation by distance relationship. It may be necessary to develop microsatellite loci with higher levels of variability to resolve this question further.

Finally, we must account for the history of drainage evolution whereby drainage-specific perturbations may affect the levels and patterns of genetic differentiation. The geomorphological history of the two drainages appears to be quite different: there is evidence that the Mulgrave and Russell rivers were mobile through much of the Pleistocene, with the mouth switching between Trinity Inlet at the north and the present mouth at Mutchero Inlet in the south. In contrast, a large basaltic lava plume flowed down the "ancestral" Johnstone River about 2 million years ago (De Keyser

1964; De Keyser and Lucas 1968), which must have greatly affected the course of this river. Given these processes, the patterns of differentiation among populations of the Mulgrave-Russell and Johnstone drainages were remarkably similar. It may be that enough time has elapsed since the perturbations for populations within each drainage to approach equilibrium. Analysis of mtDNA variation may further elucidate the importance of historical events to the extent and distribution of genetic diversity among populations of the Mulgrave-Russell and Johnstone drainages.

It is evident that populations of *P. signifer* within the Mulgrave-Russell and Johnstone drainages are not panmictic. Distance was revealed to be an important, but not exclusive, component of the variation at six allozyme loci. It may be that a more complex model, which includes populations that are in equilibrium and nonequilibrium states with other populations, may better describe the patterns of variation. It may also be that the structuring of streams, which divides populations in terms of size and probability of connectedness, may result in a complex metapopulation of interconnected demes. Future work may profit from modelling how the hierarchical structure of streams and the resultant effect on connectedness and population size can influence population structure and gene flow of obligate stream species. In addition, although allozymes provide an easy nuclear marker for analysing large numbers of samples relatively quickly, analysis of mtDNA with its smaller effective population size may be more sensitive to small population size and barriers to dispersal.

Acknowledgements

We thank the people who helped with the field collection of samples, especially Dom Loong. Brad Pusey, Mark Kennard, Steve Mackay, and Chris Marshall gave valuable advice on the location, abundance, identification, and biology of *P. signifer*. We had excellent assistance in the laboratory from Cath Cleary. Michael S. Johnson (University of Western Australia) kindly supplied a computer program that calculated pairwise G_{ST} values. Michael Arthur helped with statistical and programming concerns. Nick Campbell improved earlier drafts of this manuscript. The Co-operative Research Centre for Tropical Ecology and Management gave financial assistance to J.M.H. and to D.J.M. via a postgraduate scholarship. Griffith University contributed postgraduate research funds to D.J.M.

References

Allen, G.R. 1989. Freshwater fishes of Australia. T.F.H. Publications Inc., Neptune City, N.J.

Baer, C.F. 1998. Population structure in a south-eastern US freshwater fish, *Heterandria formosa*. II. Gene flow and biogeography within the St. Johns River drainage. Heredity, **81**: 404–411.

Blaber, S.J.M. 1980. Fish of the Trinity Inlet system of north Queensland with notes on the ecology of fish faunas of tropical Indo-Pacific estuaries. Aust. J. Mar. Freshwater Res. **31**: 137–146.

De Keyser, F. 1964. Innisfail, Qld. 1 : 250 000 Geological Series Sheet SE/55–6. Bureau of Mineral Resources, Geology and Geophysics, Canberra, Australia.

De Keyser, F., and Lucas, K.G. 1968. Geology of the Hodgkinson and Laura Basins, north Queensland. Bur. Min. Resources, Geol. Geophys. Bull., Canberra, Australia.

Guo, S.W., and Thompson, E.A. 1992. Testing differentiation in diploid populations. Biometrics, **48**: 361–372.

Hadfield, A.J., Ivanstoff, V., and Johnston, P.G. 1979. Clinal variation in electropohoretic and morphological characters between two nominal species of the genus *Pseudomugil* (Pisces: Atheriniformes: Pseudomugilidae). Aust. J. Mar. Freshwater Res. **30**: 375–386.

Hansen, M.M., Nielson, E.E., and Mensberg, K.-L.D. 1997. The problem of sampling families rather than populations: relatedness among individuals in samples of juvenile brown trout *Salmo trutta* L. Mol. Ecol. **6**: 469–474.

Hernandez-Martich, J.D., and Smith, M.H. 1997. Downstream gene flow and genetic structure of *Gambusia holbrooki* (eastern mosquitofish) populations. Heredity, **79**: 295–301.

Howe, E. 1987. Breeding behaviour, egg surface morphology and embryonic development in four species of the genus *Pseudomugil* (Pisces: Melanotaeniidae). Aust. J. Mar. Freshwater Res. **38**: 885–895.

Hughes, J.M., Bunn, S.E., Hurwood, D.A., and Cleary, C. 1998. Dispersal and recruitment of *Tasiagma ciliata* (Trichoptera: Tasimiidae) in rainforest streams, south-eastern Australia. Freshwater Biol. **39**: 117–127.

Hutchison, D.W., and Templeton, A.R. 1999. Correlation of pairwise genetic and geographic distance measures: inferring the relative influences of gene flow and drift on the influence of genetic variability. Evolution, **53**: 1898–1914.

Lu, G., Li, S., and Bernatchez, L. 1997. Mitochondrial DNA diversity, population structure, and conservation genetics of four carps within the Yangtze River, China. Can. J. Fish. Aquat. Sci. **54**: 47–58.

McGlashan, D.J. 2000. Consequences of dispersal, stream structure and earth history on patterns of allozyme and mitochondrial DNA variation of three species of Australian freshwater fish. Ph.D. thesis, Griffith University, Brisbane, Australia.

McGlashan, D.J., and Hughes, J.M. 2000. Reconciling patterns of genetic variation with stream structure, earth history and biology in the Australian freshwater fish *Craterocephalus stercusmuscarum* (Atherinidae). Mol. Ecol. **9**: 1737–1752.

Meffe, G.K., and Vrijenhoek, R.C. 1988. Conservation genetics in the management of desert fishes. Conserv. Biol. **2**: 157–169.

Nei, M. 1973. Analysis of gene diversity in subdivided populations. Proc. Natl. Acad. Sci. U.S.A. **70**: 3321–3323.

Raymond, M., and Rousset, F. 1995. GENEPOP (version 1.2). Population genetics software for exact tests and ecumenicism. J. Hered. **86**: 248–249.

Richardson, B.J., Baverstock, P.R., and Adams, M. 1986. Allozyme electrophoresis: a handbook for animal systematics and population studies. Academic Press, Sydney, Australia.

Rogers, J.S. 1972. Measures of genetic similarity and genetic distance. Studies in Genetics VII. University of Texas publication 7213. pp. 145–153.

Saeed, B., Ivantsoff, W., and Allen, G.R. 1989. Taxonomic review of the family Pseudomugilidae (Order Atheriniformes). Aust. J. Mar. Freshwater Res. **40**: 719–787.

Schmidt, S.K., Hughes, J.M., and Bunn, S.E. 1995. Gene flow among conspecific populations of *Baetis* sp. (Ephemoptera): adult flight and larval drift. J. North Am. Benthol. Soc. **14**: 147–157.

Schneider, S., Kueffer, J., Roessli, D., and Excoffier, L. 1997. Arlequin: exploratory molecular genetics software. Version 1.1. University of Geneva, Geneva, Switzerland.

Semple, G.P. 1986. *Pseudomugil signifer* — maintenance, reproduction and early development of the Pacific blue-eye. Fishes Sahul, **3**: 121–125.

Semple, G.P. 1991. Reproductive behaviour and early development of the honey blue-eye *Pseudomugil mellis* Allen and Ivanstoff

1982 (Pisces: Pseudomugilidae), from the north-east coast division, south-eastern Queensland, Australia. Aust. J. Mar. Freshwater Res. **42**: 277–286.

Shaw, P.W., Carvalho, G.R., Magurran, A.E., and Seghers, B.H. 1994. Factors affecting the distribution of genetic variability in the guppy, *Poecilia reticulata*. J. Fish Biol. **45**: 875–888.

Slatkin, M. 1977. Gene flow and genetic drift in a species subject to frequent local extinctions. Theor. Popul. Biol. **12**: 253–262.

Slatkin, M. 1987. Gene flow and the geographic structure of populations. Science (Washington, D.C.), **236**: 787–792.

Slatkin, M. 1993. Isolation by distance in equilibrium and nonequilibrium populations. Evolution, **47**: 264–279.

Appendix 1. Allele frequencies, sample sizes (*n*), and observed heterozygosity (H_O) for populations of *P. signifer* from the Mulgrave-

	Mulgrave sites									
Locus	MGA	LMB	LMA	BHB 96	BHB 97	3BC	BHA	FIG	THC	MGB
*GPI-1**										
n	16	18	37	43	44	36	42	44	43	42
2	0.219	0.111	0.054	0.093	0.148	0.069	0.060	0.068	0.035	0.012
3	0.781	0.889	0.932	0.907	0.852	0.931	0.940	0.932	0.965	0.988
5	—	—	0.014	—	—	—	—	—	—	—
H_O	0.313	0.222	0.135	0.140	0.205	0.139	0.119	0.136	0.070	0.024
*ADH**										
n	15	20	34	43	44	37	42	43	41	42
1	—	—	—	0.012	—	—	—	—	—	—
3	—	0.025	0.074	0.581	0.750	0.162	0.595	0.093	0.073	0.012
4	—	—	—	—	—	—	—	—	—	—
5	1.000	0.975	0.912	0.407	0.250	0.838	0.405	0.895	0.927	0.988
6	—	—	0.015	—	—	—	—	0.012	—	—
H_O	0.000	0.050	0.118	0.419	0.364	0.324	0.333	0.116	0.146	0.024
*PEPB**										
n	16	18	37	43	44	37	42	44	44	42
1	—	—	—	—	0.011	0.014	—	0.023	—	—
2	0.969	1.000	1.000	1.000	0.943	0.905	0.964	0.841	1.000	1.000
3	0.031	—	—	—	0.045	0.081	0.024	0.136	—	—
4	—	—	—	—	—	—	0.012	—	—	—
H_O	0.063	0.000	0.000	0.000	0.114	0.135	0.048	0.273	0.000	0.000
*PGM**										
n	15	18	37	43	44	37	40	44	43	41
2	—	—	—	—	0.011	0.068	—	—	—	0.012
4	1.000	1.000	1.000	1.000	0.989	0.932	1.000	1.000	1.000	0.988
5	—	—	—	—	—	—	—	—	—	—
H_O	0.000	0.000	0.000	0.000	0.023	0.135	0.000	0.000	0.000	0.024
*PGDH**										
n	16	19	36	43	44	37	42	44	44	41
2	0.969	1.000	1.000	0.988	1.000	0.986	0.976	1.000	1.000	1.000
3	0.031	—	—	0.012	—	0.014	0.024	—	—	—
H_O	0.063	0.000	0.000	0.023	0.000	0.027	0.000	0.000	0.000	0.000
*GPI-2**										
n	16	20	34	43	44	37	42	44	43	42
4	—	—	—	—	—	0.027	—	—	—	—
5	1.000	1.000	1.000	1.000	1.000	0.973	0.988	1.000	1.000	1.000
7	—	—	—	—	—	—	0.012	—	—	—
H_O	0.000	0.000	0.000	0.000	0.000	0.054	0.024	0.000	0.000	0.000

Note: Site abbreviations as in Fig. 1.

Spruell, P., Rieman, B.E., Knudsen, K.L., Utter, F., and Allendorf, F.W. 1999. Genetic population structure within streams: microsatellite analysis of bulltrout populations. Ecol. Freshwater Fish, **8**: 114–121.

Ward, R.D., Woodwark, M., and Skibinski, O.F. 1994. A comparison of genetic diversity levels in marine, freshwater and anadromous fishes. J. Fish Biol. **44**: 213–232.

Whitlock, M.C. 1992. Temporal fluctuations in demographic parameters and the genetic variance among populations. Evolution, **46**: 608–615.

Whitlock, M.C., and McCauley, D.E. 1990. Some population genetic consequences of colony formation and extinction: genetic correlations within founding groups. Evolution, **44**: 1717–1724.

Wright, S. 1943. Isolation by distance. Genetics, **114**: 114–138.

Russell drainage.

Russell sites							
HYC96	HYC97	DBC	RSB	DNC	RSR	CCK	WCT
43	44	43	43	44	41	36	44
—	—	0.047	0.035	—	0.037	0.056	0.091
1.000	1.000	0.953	0.965	1.000	0.963	0.944	0.909
—	—	—	—	—	—	—	—
0.000	0.000	0.093	0.070	0.000	0.073	0.056	0.136
42	44	44	43	44	40	36	43
—	—	—	—	—	—	—	—
0.017	0.036	0.148	0.221	0.239	0.425	0.417	0.267
—	—	—	—	—	—	—	0.023
0.983	0.964	0.852	0.779	0.761	0.575	0.583	0.709
—	—	—	—	—	—	—	—
0.071	0.000	0.205	0.349	0.386	0.350	0.500	0.349
44	44	44	43	44	41	34	44
—	—	—	—	—	—	—	0.011
1.000	0.989	0.977	0.837	1.000	0.963	0.956	0.955
—	0.011	0.023	0.151	—	0.037	0.044	0.034
—	—	—	0.012	—	—	—	—
0.000	0.023	0.045	0.186	0.000	0.073	0.088	0.045
44	44	44	43	44	40	38	44
—	—	—	—	—	—	—	0.045
1.000	1.000	0.977	1.000	1.000	1.000	1.000	0.955
—	—	0.023	—	—	—	—	—
0.000	0.000	0.045	0.000	0.000	0.000	0.000	0.091
44	44	44	43	44	41	37	44
0.966	1.000	1.000	1.000	1.000	1.000	1.000	1.000
0.034	—	—	—	—	—	—	—
0.068	0.000	0.000	0.000	0.000	0.000	0.000	0.000
44	44	44	43	44	41	37	44
—	—	—	—	—	—	0.014	—
1.000	1.000	1.000	1.000	1.000	1.000	0.986	1.000
—	—	—	—	—	—	—	—
0.000	0.000	0.000	0.000	0.000	0.000	0.027	0.000

Appendix 2. Allele frequencies, sample sizes (*n*), and observed heterozygosity (H_O) for populations of *P. signifer* from the Johnstone drainage.

	North Johnstone sites						South Johnstone sites					
Locus	PYC	NJT	CLC	NJN	TRC	NJD	SJR96	SJR97	MNC	UTC	STJ	MSC
*GPI-1**												
n	46	19	41	44	48	43	41	44	44	44	44	44
2	0.022	0.132	—	0.023	—	—	0.098	0.023	—	0.011	0.011	—
3	0.891	0.711	1.000	0.159	0.406	0.430	0.610	0.636	1.000	0.670	0.886	1.000
4	—	—	—	—	0.010	—	—	—	—	—	—	—
5	0.087	0.158	—	0.818	0.583	0.570	0.293	0.341	—	0.318	0.102	—
H_O	0.174	0.368	0.000	0.273	0.500	0.535	0.439	0.523	0.000	0.500	0.227	0.000
*ADH**												
n	46	19	41	44	48	44	41	44	44	44	44	44
1	0.011	—	—	—	—	—	—	—	—	0.011	—	—
3	0.272	0.342	0.012	0.102	0.104	0.159	0.098	0.068	—	0.091	0.068	—
4	—	—	0.244	0.011	—	0.080	—	—	—	—	—	—
5	0.685	0.658	0.744	0.886	0.885	0.761	0.902	0.932	1.000	0.898	0.932	1.000
6	0.033	—	—	—	0.010	—	—	—	—	—	—	—
H_O	0.239	0.474	0.171	0.182	0.188	0.295	0.195	0.136	0.000	0.182	0.136	0.000
*PEPB**												
n	48	19	38	44	47	44	42	44	44	44	44	43
1	0.021	—	—	—	0.021	0.011	0.012	0.023	—	0.011	—	—
2	0.260	0.316	1.000	0.239	0.128	0.284	0.083	0.068	—	0.102	0.136	0.570
3	0.594	0.421	—	0.761	0.606	0.670	0.833	0.807	1.000	0.830	0.841	0.430
4	0.115	0.237	—	—	0.245	0.034	0.071	0.091	—	0.057	0.023	—
6	0.010	0.026	—	—	—	—	—	0.011	—	—	—	—
H_O	0.583	0.632	0.000	0.341	0.447	0.523	0.262	0.318	0.000	0.295	0.318	0.535
*PGM**												
n	47	19	41	44	48	44	42	44	44	44	44	44
1	0.021	—	—	—	—	—	—	—	0.011	—	—	—
2	0.191	0.158	—	0.011	0.052	0.057	—	0.034	—	0.011	0.011	—
4	0.787	0.842	1.000	0.989	0.948	0.943	1.000	0.966	0.989	0.989	0.989	1.000
H_O	0.340	0.316	0.000	0.023	0.104	0.114	0.000	0.068	0.023	0.023	0.023	0.000
*PGDH**												
n	46	17	41	44	48	44	40	44	44	44	44	44
2	0.902	1.000	1.000	1.000	0.990	0.932	1.000	1.000	1.000	1.000	1.000	0.989
3	0.098	—	—	—	0.010	0.068	—	—	—	—	—	0.011
H_O	0.196	0.000	0.000	0.000	0.021	0.136	0.000	0.000	0.000	0.000	0.000	0.023
*GPI-2**												
n	48	19	41	44	48	44	42	44	44	44	44	43
4	0.010	—	—	—	—	—	—	—	—	—	—	—
5	0.979	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000
7	0.010	—	—	—	—	—	—	—	—	—	—	—
H_O	0.042	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000

Note: Site abbreviations as in Fig. 1.

Admixture analysis and stocking impact assessment in brown trout (*Salmo trutta*), estimated with incomplete baseline data

Michael M. Hansen, Einar E. Nielsen, Dorte Bekkevold, and Karen-Lise D. Mensberg

Abstract: Studies of genetic interactions between wild and domesticated fish are often hampered by unavailability of samples from wild populations prior to population admixture. We assessed the utility of a new Bayesian method, which can estimate individual admixture coefficients even with data missing from the populations contributing to admixture. We applied the method to analyse the genetic contribution of domesticated brown trout (*Salmo trutta*) in samples of anadromous trout from two stocked populations with no genetic data available before stocking. Further, we estimated population level admixture proportions by the mean of individual admixture coefficients. This method proved more informative than a multidimensional scaling analysis of individual-based genetic distances and assignment tests. The results showed almost complete absence of stocked, domesticated trout in samples of trout from the rivers. Consequently, stocking had little effect on improving fisheries. In one population, the genetic contribution by domesticated trout was small, whereas in the other population, some genetic impact was suggested. Admixture in this sample of anadromous trout despite absence of stocked domesticated trout could be because of introgression by domesticated trout adopting a resident life history.

Résumé : L'étude des interactions génétiques entre les poissons sauvages et les poissons domestiqués est souvent entravée par l'absence d'échantillons provenant de la population sauvage avant le mélange des populations. Nous avons déterminé l'utilité d'une nouvelle méthode bayésienne qui peut estimer les coefficients individuels de mélange, même lorsqu'il manque des données au sujet des populations qui ont contribué au mélange. Nous avons appliqué la méthode à l'analyse de la contribution de Truites brunes domestiquées dans des échantillon de truites anadromes de deux populations ensemencées pour lesquelles aucune information génétique préalable n'était disponible. De plus, nous avons estimé les proportions du mélange au niveau de la population par la moyenne des coefficients individuels de mélange. La méthode s'est avérée plus riche en renseignements qu'une analyse de cadrage multidimensionnel des distances génétiques calculées sur une base individuelle et des tests d'attribution. Les résultats révèlent une absence à peu près totale de truites domestiquées ensemencées dans les échantillons prélevés dans les rivières. L'ensemencement n'a donc que peu contribué à l'amélioration de la pêche. Dans l'une des populations, la contribution génétiques des truites domestiquées était faible, alors que, dans l'autre, il y avait des signes d'un impact génétique. L'existence d'un mélange génétique dans cette population de truites anadromes, malgré l'absence de truites domestiquées ensemencées, pourrait être due à une introgression à partir de truites domestiquées qui ont adopté un cycle biologique de type sédentaire.

[Traduit par la Rédaction]

Introduction

Salmonid fishes are one of the groups of organisms in which most translocations and gene flow from captive to wild populations have occurred owing to unintentional escapes or deliberate releases of exogenous and often domesticated fish (Hindar et al. 1991*a*; Hansen and Loeschcke 1994; Laikre 1999). Estimation of the genetic impact of domesticated fish on wild populations has been the focus of several studies. Controlled stocking experiments have demonstrated poor performance of domesticated fish in the wild (e.g., McGinnity et al. 1997; Fleming et al. 2000). However, in some parts of their life cycle, domesticated fish appear to have a competitive advantage over wild fish, presumably because of higher growth rates (McGinnity et al. 1997; Fleming et al. 2000). In the latter two studies, the performance of F1 "hybrids" was mainly intermediate relative to the parental wild and domesticated populations, but the performance of F2 and further generations was not assessed, presumably because of the long time scale required for such studies. One way to assess the long-term genetic impact of domesticated fish involves the use of genetic markers for opportunistic screening of wild populations subject to stocking or escapes of domesti-

Received March 9, 2001. Accepted July 27, 2001. Published on the NRC Research Press Web site at http://cjfas.nrc.ca on August 30, 2001.
J16252

M.M. Hansen,[1] E.E. Nielsen, and K.-L.D. Mensberg. Danish Institute for Fisheries Research, Department of Inland Fisheries, Population Genetics Laboratory, Vejlsøvej 39, DK-8600 Silkeborg, Denmark.
D. Bekkevold. Institute of Biological Sciences, Department of Ecology and Genetics, Ny Munkegade, Building 540, University of Aarhus, DK-8000 Århus C, Denmark.

[1] Corresponding author (e-mail: mmh@dfu.min.dk).

DOI: 10.1139/cjfas-58-9-1853

cated fish. Most studies of this kind have confirmed a decreased performance of domesticated fish (e.g., Moran et al. 1991; Poteaux et al. 1998; Hansen et al. 2000). At the same time, it has been documented that domesticated and wild fish do interbreed (e.g., Largiadèr and Scholl 1996; Hansen et al. 2000). This raises a question as to the extent to which domesticated fish cause permanent introgression into wild gene pools.

In some cases (e.g., Moran et al. 1991; Largiadèr and Scholl 1996), diagnostic genetic markers are available in wild and domesticated populations, respectively, that may be used for estimating the genetic contribution of domesticated fish. However, in other studies, a fundamental problem is that data are not available from populations before possible genetic impact took place (e.g., Hansen et al. 2001*a*). Analysis of DNA from historical samples (for instance, old scale samples) could be used as baseline data for populations before possible population admixture (Nielsen et al. 1997, 2001). However, if historical samples are not available, analyses must rely on samples from contemporary and possibly admixed populations. In this case, methods for assigning individuals to populations, so-called assignment tests (Paetkau et al. 1995), can be used as rough indicators of whether domesticated fish have made a significant genetic contribution (Hansen et al. 2001*a*; Ruzzante et al. 2001). It remains problematic, however, that domesticated fish within the samples of wild-caught fish may bias allele frequencies and, consequently, assignment accuracy (Hansen et al. 2001*b*).

Recently, a new model-based Bayesian method has been developed (Pritchard et al. 2000) that, among other things, can be used to estimate the posterior distribution of individual admixture coefficients, i.e., the proportion of an individual's genome that is derived from one or the other population. This can be done even in the absence of samples from the parental populations, i.e., it is possible to estimate individual admixture coefficients solely from a mixed sample, though it is obviously an advantage if samples from parental populations can be used for assisting in the estimations. This type of analysis was recently used in a study of introgression by domestic cat (*Felis catus*) into Scottish wild cat (*F. silvestris*) gene pools (Beaumont et al. 2001). No samples were available from known non-introgressed wild cats, but by using a model assuming two populations and analysing a sample of known domestic cats and a presumably mixed sample of wild and domestic cats, it was possible to estimate admixture coefficients of individuals in the mixed sample and assess the overall introgression status of wild cats.

The design of the study by Beaumont et al. (2001) closely resembles the situation in studies of introgression in salmonid fishes with no baseline samples available from populations before genetic impact. Therefore, in the present study, we wanted to explore the usefulness of estimating individual admixture coefficients for assessing the impact of stocked domesticated brown trout (*Salmo trutta*) on wild brown trout populations. Furthermore, the mean of individual admixture coefficients within a sample provides an estimate of population level admixture, and we wanted to explore this possibility for estimating admixture proportions in a mixed sample with incomplete baseline data. For comparison, we also performed assignment tests (Paetkau et al. 1995; Cornuet et al. 1999) and multidimensional scaling analyses based on genetic distances between individuals. We studied two stocked populations, one of which had previously been suggested to be virtually unaffected by domesticated trout based on microsatellite analysis (Hansen et al. 2001*a*). We wanted to reassess this conclusion using individual admixture analysis. The other population sampled was still being intensively stocked with domesticated trout when samples were taken. Fisheries managers have claimed that the present run of anadromous trout in the river was an example of a successful outcome of stocking with domesticated trout, as natural production in the river was thought to be minimal relative to the number of stocked fish. Therefore, we wanted to assess the extent to which domesticated fish contributed to the rehabilitation of the river population.

Materials and methods

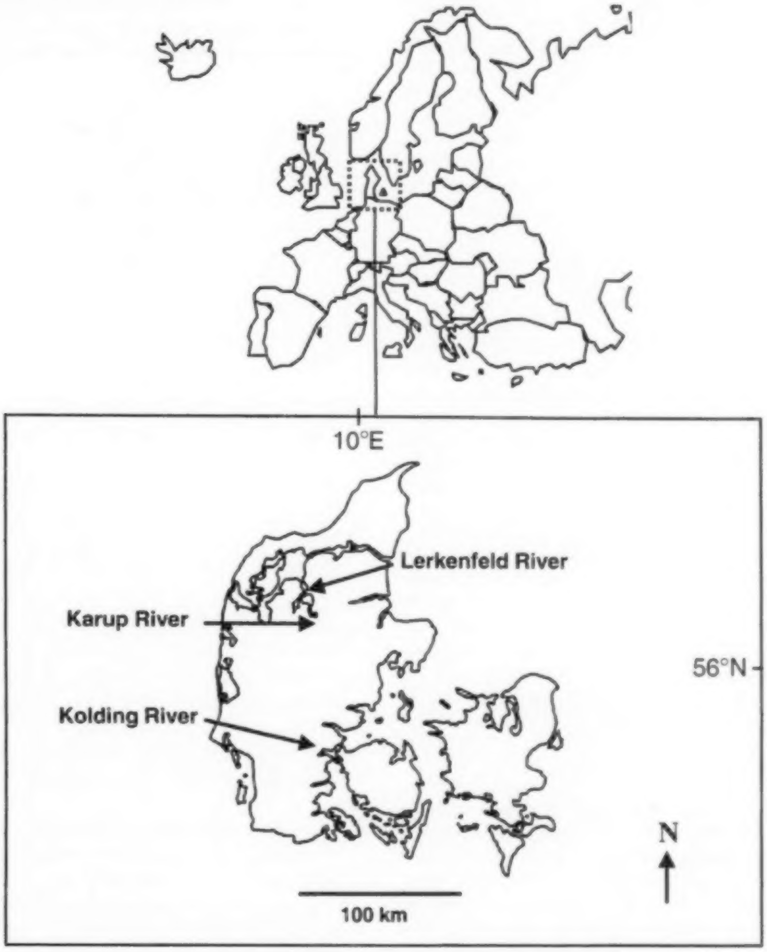
Populations studied

Brown trout is an iteroparous salmonid species naturally distributed in Europe, North-West Asia, and North Africa (Elliott 1994). In the Atlantic region, the species exhibits both resident and migratory (including anadromous) life-history forms. Most brown trout populations in Denmark consist of a mixture of resident and anadromous trout (sea trout), which presumably interbreed freely (Hindar et al. 1991*b*). We studied two neighbouring brown trout populations that both have a long history of stocking with domesticated trout from one particular strain (Hårkær Hatchery). The Lerkenfeld River (see Fig. 1) is presently stocked with 42 000 domesticated trout per year (of age classes 0+, 1+, and 2+), and by accounting for the ages of stocked fish, assuming that survival at different ages equals that of wild trout, and by comparing the number of stocked trout to the estimated natural production, the number of domesticated trout should be 2–5 times higher than naturally produced trout. We analysed a sample of 37 adult sea trout spawners from the river, caught by electrofishing in the main river during the spawning season in November 1999 (sample abbreviation LER). The Karup River population (sample abbreviation KAR, 48 anadromous trout sampled during the spawning season in November 1993) is presently stocked exclusively with trout of local origin (supportive breeding), but until the early 1990s, it was heavily stocked with Hårkær strain trout. Previous studies (Hansen et al. 2001*a*) suggested that domesticated trout have not significantly affected the present gene pool, although some interbreeding was observed in a localised area where stocking was still ongoing (Hansen et al. 2000). We wanted to reassess the conclusion of a limited genetic contribution by domesticated trout using the methods by Pritchard et al. (2000). Finally, we analysed 88 individuals from the Hårkær Hatchery strain (HAT), collected in 1993 and 1994. The strain is of partly unknown origin but has presumably been founded mainly by trout from the Kolding River (Fig. 1) at least some 50 years ago (Hansen et al. 2001*a*).

Microsatellite analysis

DNA was extracted from frozen muscle or adipose fin tissue, stored in ethanol, using phenol–chloroform extraction (Taggart et al. 1992) or proteinase K – Chelex extraction protocols (Estoup et al. 1996). One tetranucleotide and eight dinucleotide microsatellite loci were analysed: Str 15, Str 60, Str 73 (Estoup et al. 1993), Ssa 85, Ssa 197 (tetranucleotide microsatellite) (O'Reilly et al. 1996), SsoSL 417 (Slettan et al. 1995), SsoSL 438 (Slettan et al. 1996), T3-13 (Estoup et al. 1998), and SsHaeIII.14.20 (J.L. Goodier, Department of Biochemistry, Memorial University of Newfoundland, St. John's, NF A1B 3X9, Canada, unpublished data; GenBank accession number U10050). One primer of each primer set was end-labelled with the fluorescent dye CY5, and the microsatellites were

Fig. 1. Map showing the approximate locations of sampling sites in Denmark. The Kolding River, the presumed locality of origin of the Hårkær Hatchery trout, is also indicated.



analysed on a Pharmacia ALFexpress automated sequencer, according to the manufacturer's recommendations. Gels were scored using ALLELINKS vers. 1.00 (Pharmacia).

Statistical analysis

Deviations from Hardy–Weinberg equilibrium were tested using exact tests (Guo and Thompson 1992). Genetic differentiation between pairs of populations was estimated with pairwise θ estimates (Weir and Cockerham 1984), and their significances were tested by permuting individuals between samples. These tests and analyses were done using the program ARLEQUIN 1.1 (Schneider et al. 1997).

The genetic relationships among individuals from HAT, LER, and KAR were analysed by calculating genetic distances between all individuals (–ln(proportion of alleles shared between individuals); Bowcock et al. 1994) and visualising the groupings of individuals by multidimensional scaling, using the program VISTA 5.6.3 (Young 1996).

For assigning individuals to populations, we used an assignment test where individuals are assigned to the population from which they have the highest likelihood of being derived, based on individual multilocus genotypes and allele frequencies of population samples (Paetkau et al. 1995). We used a modified version of the assignment test in which population allele frequencies are estimated using a Bayesian approach. These analyses were performed using the software GeneClass 1.0 (Cornuet et al. 1999). We also used the "simulation" option of GeneClass for assessing the probability of a multilocus genotype being derived from the allele frequencies of a population, which makes it possible to "accept" or "reject" individuals in populations. A number of multilocus genotypes (in this case 10 000) are randomly generated, based on the estimates of allele frequencies of the specific population, and a frequency distribution of likelihood values is generated. The likelihood of an individual can then be compared to this distribution, and if the value is below a certain threshold probability level, in this case 5%, the individual is "rejected" from the sample. We performed the assignment tests involving all three populations at the same time.

Individual admixture coefficients and the numbers of populations represented by the LER and KAR samples were estimated using Structure (Pritchard et al. 2000). This is a Bayesian Markov Chain Monte Carlo (MCMC) based approach that uses model-based clustering for partitioning all individuals into groups, and the criteria for grouping individuals are to minimise Hardy–Weinberg disequilibrium and gametic phase disequilibrium between loci within groups. This ensures that the groups are as representative as possible of samples from single populations as Hardy–Weinberg disequilibrium and gametic phase disequilibrium would be expected if groups consisted of individuals from different populations. Structure allows for several kinds of analyses, including assigning individuals to populations, estimating allele frequencies of populations taking admixture between populations into account, estimating the number of populations represented by a sample of individuals, and estimating individual admixture coefficients. In this study we used the latter two options. First, we estimated the number of populations represented by the samples of wild trout (LER and KAR) that were potentially admixed owing to stocking. This was done by estimating the probability of the data assuming a model in which the sampled individuals represented one, two, or three populations, and then the relative probability for each number of populations was calculated, as described in Pritchard et al. (2000). In practice, this was done by analysing the LER and KAR samples separately assuming one, two, or three populations and using a burn-in period of 200 000 steps followed by 1 000 000 MCMC replicates. The posterior distribution of individual admixture coefficients (q) and their 90% probability intervals were estimated by assuming an admixture model with two populations, i.e., HAT and either the LER or KAR population. As recommended by Pritchard et al. (2000) for weakly differentiated populations, we used a model in which allele frequencies in populations were correlated. Furthermore, as gene flow could not possibly have occurred from LER or KAR to HAT, we forced the model to consider all HAT individuals as being nonadmixed. We used a burn-in period of 50 000 steps and then proceeded with 100 000 MCMC replicates.

The mean individual q value within a sample is an estimate of the total genic admixture proportions. It is therefore possible to estimate admixture proportions within a sample with incomplete or missing baseline data from the parental populations. We estimated population-level admixture in LER and KAR by mean q, and to estimate 95% confidence intervals, we calculated mean q from bootstrapped samples from the posterior distribution of q values. In other words, there are two sources of variation: variation among individuals and variation in q values in different MCMC replicates. To have both sources of variation represented, we sampled individual q values from 20 single steps of the Markov Chain (i.e., a burn-in period of 50 000 steps followed by just one MCMC replicate), drew 10 000 samples (of size n) from these pooled q values, and estimated confidence intervals from the distribution of mean q values.

Table 1. Summary of microsatellite data: observed number of alleles per locus and sample, outcome of tests for deviations from expected Hardy–Weinberg (H–W) proportions, and expected (H_e) and observed heterozygosity (H_o).

Microsatellite locus		HAT	LER	KAR
Str 15	No. alleles	4	4	5
(total no.	H–W test	ns	ns	ns
alleles: 5)	H_e	0.67	0.66	0.71
	H_o	0.61	0.65	0.69
Str 73	No. alleles	4	3	3
(total no.	H–W test	ns	ns	ns
alleles: 4)	H_e	0.59	0.60	0.50
	H_o	0.58	0.46	0.50
Str 60	No. alleles	3	3	3
(total no.	H–W test	ns	ns	ns
alleles: 3)	H_e	0.40	0.37	0.54
	H_o	0.41	0.41	0.52
SsoSL 417	No. alleles	10	9	8
(total no.	H–W test	ns	ns	ns
alleles: 11)	H_e	0.80	0.84	0.76
	H_o	0.73	0.76	0.75
SsoSL 438	No. alleles	5	4	5
(total no.	H–W test	*	ns	***
alleles: 6)	H_e	0.72	0.62	0.59
	H_o	0.56	0.65	0.73
Ssa 85	No. alleles	5	6	6
(total no.	H–W test	ns	ns	ns
alleles: 7)	H_e	0.65	0.74	0.75
	H_o	0.64	0.76	0.79
Ssa 197	No. alleles	7	9	10
(total no.	H–W test	**	ns	ns
alleles: 10)	H_e	0.78	0.84	0.75
	H_o	0.80	0.84	0.77
T3–13	No. alleles	17	17	17
(total no.	H–W test	ns	ns	ns
alleles: 21)	H_e	0.80	0.91	0.90
	H_o	0.83	0.95	0.88
SsHaeIII14.20	No. alleles	9	10	8
(total no.	H–W test	ns	ns	ns
alleles: 11)	H_e	0.76	0.81	0.83
	H_o	0.76	0.70	0.85

Note: HAT denotes the Hårkær Hatchery strain, LER denotes the Lerkenfeld River population, and KAR denotes the Karup River population. Table-wide significance levels were applied, using the sequential Bonferroni technique (Rice 1989) ($k = 27$). ns, not significant; *, significant at the 5% level; **, significant at the 1% level; ***,significant at the 0.1% level.

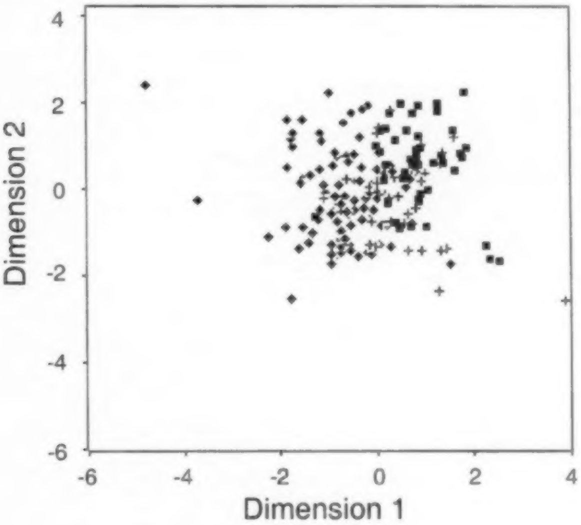
Results

The number of alleles per locus in populations varied from three at the locus Str 60 to 17 at the locus T3-13 (Table 1). Three significant deviations from Hardy–Weinberg equilibrium were observed, two of them at the locus SsoSL 438. However, deviations were not likely to reflect null alleles as one deviation was associated with a heterozygote deficiency and the other with a heterozygote excess. θ was 0.04 between HAT and LER, 0.06 between HAT and KAR, and 0.02 between LER and KAR. All values were significant at the 0.1% level.

The multidimensional scaling analysis of genetic distances between individuals (Fig. 2) showed some separation among individuals from HAT and wild trout (LER and KAR). However, there was considerable overlap between these two groups, and it was not possible to separate LER and KAR trout. Dimensions 1 and 2 represented only 5.6% and 5.1% of the total variance, respectively, but inspection of all scatter plots involving combinations of the first five dimensions yielded essentially the same result. Also, factorial correspondence analysis yielded similar results (results not shown).

Using GeneClass, a high proportion of individuals from HAT was assigned correctly (Table 2). Relatively lower proportions of trout from LER and KAR were assigned to their sample of origin, but nearly all misassigned individuals from LER were assigned to KAR and vice versa. Only two individuals from each of LER and KAR were assigned to HAT. Using the "simulation option" of GeneClass, a large proportion of HAT individuals was rejected from KAR and vice versa (Table 2). Conversely, fewer individuals from LER were rejected in KAR and vice versa. A relatively small pro-

Fig. 2. Scatter plot of multidimensional scaling analysis of genetic distances between individuals (Bowcock et al. 1994) from the HAT (◆), KAR (+), and LER (■) samples. Dimension 1 represents 5.6%, and dimension 2, 5.1% of the total variance.



portion of HAT trout (0.19) were rejected in LER, whereas a large proportion (0.86) of LER trout were rejected in HAT. This could be the result of unidirectional gene flow from HAT to LER owing to stocking, which could introduce allelic variation in LER that would make HAT trout more "acceptable" in LER, whereas the lack of gene flow from LER to HAT would still result in rejection of a large proportion of LER genotypes in HAT.

The estimates of the numbers of populations represented by KAR and LER suggested in both cases that one population was present. In LER, the probability of the presence of one population was 0.999, the probability of two populations 0.001, and the probability of three populations was virtually zero. In KAR, the probability of the individuals representing one population was virtually 1. For visualising individual admixture coefficients, q, we ranked individual values and plotted rank versus individual q values including 90% probability intervals (Fig. 3). A q value of 0 would denote a pure HAT trout, whereas a value of 1 would denote a pure indigenous trout genotype. All probability intervals for q values included 0, 1, or both. Therefore, it was not possible to unambiguously identify single admixed individuals as individuals with intermediate q values and probability intervals ranging from 0 to 1 could either be admixed or just exhibit uninformative genotypes. The results from KAR (Fig. 3*a*) showed that 39 of the 48 individuals exhibited q values ranging from 0.90 to 0.99 and most of those exhibited reasonably narrow probability intervals. Only two individuals were more likely of partly or fully domesticated rather than indigenous origin. Eleven individuals exhibited very wide probability intervals, in some cases ranging from 1 to 0, and this group could include admixed or domesticated trout, though it is also possible that some or all of them just exhibited noninformative genotypes. The distribution of q values in LER showed a different pattern (Fig. 3*b*). Only one individual was more likely of domesticated than indigenous origin; however, compared with KAR, q values were lower and most probability intervals included both 0 and 1. Ten individuals exhibited q values between 0.90 and 0.96, whereas in 26 of the individuals, q ranged from 0.60 to 0.89. In total, this suggests the presence of at least some admixed individuals though, on the other hand, there were few indications of the presence of "pure" domesticated trout in the LER sample. A further analysis assuming three instead of two populations did not result in better resolution and more narrow probability intervals, showing that further substructuring within the LER population was not an explanation of the wide probability intervals (data not shown). This was also consistent with the high probability of the individuals representing one population.

The estimates of population-level admixture suggested that HAT trout had made a genetic contribution to the KAR population of 0.08 (95% confidence interval (CI) 0.02–0.14). The estimated proportion of domesticated trout in LER was higher, i.e., 0.18 (95% CI 0.07–0.29).

Discussion

Individual-based statistics for analysing population admixtures

Individual-based statistics, in particular assignment tests (Paetkau et al. 1995), for determining the population of origin of individuals are being applied increasingly in fish population genetics studies and in practical management of populations (Hansen et al. 2001*b*). Of the three approaches used in this study, multidimensional scaling analysis of individual-based genetic distances clearly provided the lowest resolution. The fact that the distribution of individuals from HAT and from the two presumably mixed samples from LER and KAR overlapped could be interpreted as population admixture owing to stocking, but it could also be a result of the relatively low genetic differentiation among populations.

Assignment tests can be used as rough indicators of the degree of admixture when genetic data from wild populations before admixture are not available (Hansen et al. 2001*b*). A high proportion of individuals from the wild population assigned to the domesticated strain may indicate a significant genetic impact. Conversely, a low proportion of wild individuals assigned to the domesticated strain would suggest limited or no impact. However, as the sample from the "wild" population may consist of both wild and a number of domesticated fish, allele frequencies may be biased towards the allele frequencies of the sample of domesticated fish. Therefore, the actual contribution of domesticated fish in the sample of wild fish will be underestimated as a number of domesticated fish within the sample of wild fish will tend to be assigned to this sample instead of their true population of origin. In the present study, assignment tests indicated that the contribution of domesticated fish in LER and KAR was small. However, it was unclear the extent to which the low proportions of individuals from LER and KAR assigned to HAT were representative of the admixture that had taken place, or if admixture had in fact been underestimated. The simulation tests (Cornuet et al. 1999) added some more information as the low proportion of HAT trout rejected in LER

Table 2. Results of assignment tests, using the Bayesian approach included in the program GeneClass 1.0 (Cornuet et al. 1999).

Sample	HAT	LER	KAR	Rejected from all
HAT assigned to	**78 (0.89)**	8 (0.09)	2 (0.01)	
HAT rejected from	**11 (0.13)**	17 (0.19)	68 (0.77)	7 (0.08)
LER assigned to	2 (0.05)	**26 (0.70)**	9 (0.24)	
LER rejected from	32 (0.86)	**6 (0.16)**	13 (0.35)	4 (0.11)
KAR assigned to	2 (0.04)	9 (0.19)	**37 (0.77)**	
KAR rejected from	42 (0.88)	13 (0.28)	**6 (0.13)**	5 (0.10)

Note: "Rejected" denotes the proportion of individuals with multilocus genotypes that were among the 5% most unlikely genotypes in one of the two baseline samples. Absolute numbers and frequencies (in parentheses) are listed, and numbers in bold denote the proportion of individuals assigned to or rejected from their sample of origin. HAT denotes the Hårkær Hatchery strain, LER denotes the Lerkenfeld River population, and KAR denotes the Karup River population.

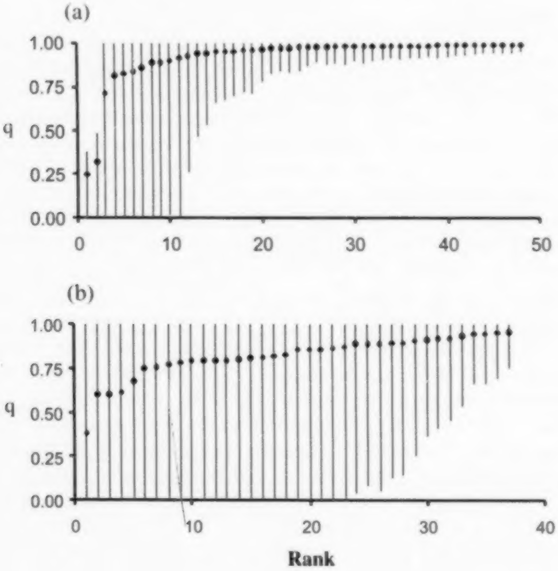


Fig. 3. Plots of individual admixture coefficients (q), including their 90% probability limits for individuals from (a) the Karup River (KAR) and (b) the Lerkenfeld River (LER). A q value of 0 denotes a "pure" domesticated trout, whereas 1 denotes a "pure" indigenous trout. Individuals were ranked from lowest to highest q value and rank was plotted against q. Estimation of q was done using the methods and Structure software by Pritchard et al. (2000).

indicated that some genetic influence by HAT trout had in fact taken place in this population.

Compared with multidimensional scaling of individual distances and assignment tests, the analyses of individual admixture coefficients proved highly informative. The large proportion of individuals with high q values and narrow probability intervals observed in KAR documented the presence of many non-admixed individuals in this population. Conversely, the lower q values of LER individuals suggested that some admixture had taken place, even though just one individual had a q value below 0.5. However, the very wide posterior probability intervals observed for individuals with intermediate admixture coefficients (q) made it difficult to identify single admixed individuals, both in KAR and in LER. This property was pointed out by Pritchard et al. (2000) and was also a problem in the study by Beaumont et al. (2001), who only observed a few individuals with intermediate q values where probability limits did not range from 0 to 1, despite θ values of up to 0.14 between groups of domesticated and putative wild cats. Both in this study and in the present one, nine microsatellite loci were analysed, but even more loci are clearly needed for reliable identification of single admixed individuals.

The population level admixture coefficients estimated by the mean of individual q values also provided highly informative results and, in this case, with considerably narrower confidence intervals compared with individual q values. This provides a method for estimating admixture proportions in populations affected by exogenous fish without knowledge of the original genetic composition of the indigenous populations, which is usually the case for stocked salmonid populations. It should be noted that other methods for mixed-stock analysis have been developed that also allow for estimating admixture proportions with missing baseline data (Smouse et al. 1990), including a newly developed Bayesian method (Pella and Masuda 2001).

In conclusion, we found that estimating individual and population level admixture using the Structure approach by Pritchard et al. (2000) proved useful for analysing the genetic contribution of stocked domesticated trout in wild populations. This method made it possible to circumvent the problem of not having baseline data from the populations before stocking and having both stocked domesticated and wild fish, including admixed individuals, in the same sample. Also, the possibility of not only assigning individuals to populations but also of assessing whether or not they were admixed was particularly useful in the context of stocking impact assessment where interbreeding between wild and stocked fish is expected to take place.

Conservation and management implications

The purpose of stocking fish populations is often unclear. Sometimes stocking activity is claimed to serve conservation purposes, but in reality, most stocking programmes are intended for improving fisheries (Laikre 1999). If we consider the outcome of stocking domesticated trout into the Karup and Lerkenfeld Rivers from a fisheries' perspective, the stocking programmes appear to have had limited effect. This is particularly obvious in the Lerkenfeld River where stocking of domesticated trout was still ongoing at the time of sampling and the number of stocked trout was expected to be several times higher than the number of naturally produced trout. Our results showed that there were few, if any, individuals of "pure" domesticated origin in a sample comprising 37 anadromous trout taken from this river, meaning that the stocked domesticated trout could not have made a significant contribution to the run of anadromous trout. However, we cannot rule out that stocked domesticated trout adopting a resident rather than anadromous life history could have made a contribution to the fisheries (see below).

From a conservation perspective, past stocking of domesticated trout from the Hårkær Hatchery strain has contributed little to rehabilitating the KAR population. A higher and non-negligible genetic contribution from domesticated trout was suggested in LER, although indigenous trout clearly made up most of the gene pool. This finding of some genetic contribution from domesticated trout is interesting, given that there were few indications of the presence of stocked domesticated trout in the sample. One explanation could be that introgression had occurred in the past because of one or a few years of high survival and reproductive success of stocked domesticated trout, whereas in other years, survival of stocked trout was low, as indicated by the analysed sample. Alternatively, life-history variation could play a role. Hansen et al. (2000) demonstrated a significant proportion of domesticated trout and interbreeding between domesticated and wild trout among resident trout in the Karup River, whereas there were few signs of domesticated individuals among anadromous trout. This suggests poor performance of domesticated trout, which become anadromous compared with resident domesticated trout, an hypothesis that is also supported by the general conclusion in a recent review by Utter (2000), who found more susceptibility to introgression in resident relative to anadromous populations in several salmonid species. Even though we did not sample resident trout from the Lerkenfeld River, a similar situation could have occurred in this population with some spawning intrusion from resident HAT trout. This could then explain the apparent genetic contribution by HAT trout among anadromous trout and at the same time the finding of only few individuals likely to be stocked domesticated trout. Finally, the possibility should be considered that the suggested contribution of HAT trout in LER is simply an artefact as the result of heterogeneity within the sample, causing increased Hardy–Weinberg and gametic phase disequilibrium. We find this explanation less likely because of the high probability of the sample representing just one population. On the other side, this result would also contradict the presence of HAT trout within the sample, but this could be explained by the almost complete absence of individuals suggested to be "pure" HAT trout. The presence of some admixed individuals combined with the relatively weak genetic differentiation between HAT and LER may be insufficient to result in detectable disequilibria within the sample, whereas the addition of HAT as a baseline sample for the estimation of individual admixture coefficients provides increased resolution. The presence of a genetic contribution by HAT in the LER sample is also supported by the assignment tests where most HAT trout were "accepted" in LER, whereas most LER trout were "rejected" in HAT.

The genetic contribution by HAT trout in LER is too small to suggest that this has contributed to the rehabilitation of this population. In fact, the admixture by HAT trout is more likely to have negative consequences. If there is natural selection acting against domesticated trout and admixed individuals as suggested by several studies (e.g., Fleming et al. 2000; Poteaux et al. 1998; Hansen et al. 2000), then spawning intrusion by domesticated trout into wild gene pools would be expected to cause a fitness decrease of the wild populations. It is becoming an increasingly accepted consensus that stocking wild populations with non-native domesticated fish is not a viable conservation option. Instead, current management guidelines suggest that efforts should focus on improving environmental conditions, possibly supplemented with supportive breeding of local populations (e.g., Laikre 1999).

Acknowledgements

We thank Daniel Ruzzante and two anonymous reviewers for useful comments and suggestions on the paper, Jonathan Pritchard for help and advice on the use of Structure and the anglers' clubs at the Lerkenfeld and Karup rivers for collection of samples.

References

Beaumont, M., Barratt, E.M., Gotelli, D., Kitchener, A.C., Daniels, M.J., Pritchard, J.K., and Bruford, M.W. 2001. Genetic diversity and introgression in the Scottish wildcat. Mol. Ecol. **10**: 319–336.

Bowcock, A.M., Ruiz-Linares, A., Tomfohrde, J., Minch, E., Kidd, J.R., and Cavalli-Sforza, L.L. 1994. High resolution of human evolutionary trees with polymorphic microsatellites. Nature, **368**: 455–457.

Cornuet, J.M., Piry, S., Luikart, G., Estoup, A., and Solignac, M. 1999. Comparison of methods employing multilocus genotypes to select or exclude populations as origins of individuals. Genetics, **153**: 1989–2000.

Elliott, J.M. 1994. Quantitative ecology and the brown trout. Oxford University Press, Oxford, UK.

Estoup, A., Presa, P., Krieg, F., Vaiman, D., and Guyomard, R. 1993. $(CT)_n$ and $(GT)_n$ microsatellites: a new class of genetic markers for *Salmo trutta* L. (brown trout). Heredity, **71**: 488–496.

Estoup, A., Largiader, C.R., Perrot, E., and Chourrout, D. 1996. Rapid one-tube DNA extraction for reliable PCR detection of fish polymorphic markers and transgenes. Mol. Mar. Biol. Biotechnol. **5**: 295–298.

Estoup, A., Rousset, F., Michalakis, Y., Cornuet, J.-M., Adriamanga, M., and Guyomard, R. 1998. Comparative analysis of microsatellite and allozyme markers: a case study investigating microgeographic differentiation in brown trout (*Salmo trutta*). Mol. Ecol. **7**: 339–353.

Fleming, I.A., Hindar, K., Mjolnerod, I.B., Jonsson, B., Balstad, T., and Lamberg, A. 2000. Lifetime success and interactions of farm salmon invading a native population. Proc. Roy. Soc. Lond. B, **267**: 1517–1523.

Guo, S.W., and Thompson, E.A. 1992. Performing the exact test for Hardy–Weinberg proportion for multiple alleles. Biometrics, **48**: 361–372.

Hansen, M.M., and Loeschcke, V. 1994. Effects of releasing hatchery-reared brown trout to wild trout populations. *In* Conservation genetics. *Edited by* V. Loeschcke, J. Tomiuk, and S.K. Jain. Birkhäuser Verlag, Basel. pp. 273–289.

Hansen, M.M., Ruzzante, D.E., Nielsen, E.E., and Mensberg, K.-L.D. 2000. Microsatellite and mitochondrial DNA polymorphism reveals life-history dependent interbreeding between hatchery and wild brown trout (*Salmo trutta* L.). Mol. Ecol. **9**: 583–594.

Hansen, M.M., Ruzzante, D.E., Nielsen, E.E., and Mensberg, K.-L.D. 2001*a*. Brown trout (*Salmo trutta*) stocking impact assessment using microsatellite DNA markers. Ecol. Appl. **11**: 148–160.

Hansen, M.M., Kenchington, E., and Nielsen, E.E. 2001*b*. Assigning individual fish to populations using microsatellite markers: methods and applications. Fish Fish. **2**: 93–112.

Hindar, K., Ryman, N., and Utter, F.M. 1991*a*. Genetic effects of cultured fish on natural fish populations. Can. J. Fish. Aquat. Sci. **48**: 945–957.

Hindar, K., Jonsson, B., Ryman, N., and Ståhl, G. 1991*b*. Genetic relationships among landlocked, resident, and anadromous brown trout, *Salmo trutta* L. Heredity, **66**: 83–91.

Laikre, L. (*Editor*). 1999. Conservation genetic management of brown trout (*Salmo trutta*) in Europe. Report by the Concerted action on identification management and exploitation of genetic resources in brown trout (*Salmo trutta*). ("Troutconcert"; EU FAIR CT97-3882). http://www.dfu.min.dk/ffi/consreport/index.htm

Largiadèr, C.R., and Scholl, A. 1996. Genetic introgression between native and introduced brown trout (*Salmo trutta* L.) populations in the Rhône River Basin. Mol. Ecol. **5**: 417–426.

McGinnity, P., Stone, C., Taggart, J.B., Cooke, D., Cotter, D., Hynes, R., McCamley, C., Cross, T., and Ferguson, A. 1997. Genetic impact of escaped farmed Atlantic salmon (*Salmo salar* L.) on native populations: use of DNA profiling to assess freshwater performance of wild, farmed, and hybrid progeny in a natural river environment. ICES J. Mar. Sci. **54**: 998–1008.

Moran, P., Pendas, A.M., Garcia-Vazquez, E., and Izquierdo, J. 1991. Failure of a stocking policy of hatchery reared brown trout, *Salmo trutta* L., in Asturias, Spain, detected using *LDH-5** as a genetic marker. J. Fish Biol. **39**(Suppl. A): 117–121.

Nielsen, E.E., Hansen, M.M., and Loeschcke, V. 1997. Analysis of microsatellite DNA from old scale samples of Atlantic salmon: a comparison of genetic composition over sixty years. Mol. Ecol. **6**: 487–492.

Nielsen, E.E., Hansen, M.M., and Bach, L. 2001. Looking for a needle in a haystack: discovery of indigenous salmon in heavily stocked populations. Conserv. Genet. In press.

O'Reilly, P.T., Hamilton, L.C., McConnell, S.K., and Wright, J.M. 1996. Rapid analysis of genetic variation in Atlantic salmon (*Salmo salar*) by PCR multiplexing of dinucleotide and tetranucleotide microsatellites. Can. J. Fish. Aquat. Sci. **53**: 2292–2298.

Paetkau, D., Calvert, W., Stirling, I., and Strobeck, C. 1995. Microsatellite analysis of population structure in Canadian polar bears. Mol. Ecol. **4**: 347–354.

Pella, J., and Masuda, M. 2001. Bayesian methods for analysis of stock mixtures from genetic characters. Fish. Bull. **99**: 151–167.

Poteaux, C., Beaudou, D., and Berrebi, P. 1998 Temporal variations of genetic introgression in stocked brown trout populations. J. Fish Biol. **53**: 701–713.

Pritchard, J.K., Stephens, M., and Donnelly, P. 2000. Inference of population structure using multilocus genotype data. Genetics, **155**: 945–959.

Rice, W.R. 1989. Analyzing tables of statistical tests. Evolution, **43**: 223–225.

Ruzzante, D.E., Hansen, M.M., and Meldrup, D. 2001. Distribution of individual inbreeding coefficients, relatedness and influence of stocking on native anadromous brown trout (*Salmo trutta*) population structure. Mol. Ecol. **10**: 2107–2128.

Schneider, S., Kueffer, J.-M., Roessli, D., and Excoffier, L. 1997. Arlequin. Version 1.1. Genetics and Biometry Laboratory, Department of Anthropology, University of Geneva, Switzerland.

Slettan, A., Olsaker, I., and Lie, Ø. 1995. Atlantic salmon, *Salmo salar*, microsatellites at the SSOSL25, SSOSL85, SSOSL311, SSOSL417 loci. Anim. Genet. **26**: 277–285.

Slettan, A., Olsaker, I., and Lie, Ø. 1996. Atlantic salmon, *Salmo salar*, microsatellites at the SSOSL438, SSOSL439 and SSOSL444 loci. Anim. Genet. **27**: 57–64.

Smouse, P.E., Waples, R.S., and Tworek, J.A. 1990. A genetic mixture analysis for use with incomplete source population data. Can. J. Fish. Aquat. Sci. **47**: 620–634.

Taggart, J.B., Hynes, R.A., Prodöhl, P.A., and Ferguson, A. 1992. A simplified protocol for routine total DNA isolation from salmonid fishes. J. Fish Biol. **40**: 963–965.

Utter, F.M. 2000. Patterns of subspecific anthropogenic introgression in two salmonid genera. Rev. Fish. Fish. Biol. **10**: 265–279.

Weir, B.S., and Cockerham, C.C. 1984. Estimating F-statistics for the analysis of population structure. Evolution, **38**: 1358–1370.

Young, F.W. 1996. ViSta: the Visual Statistics System. Research Memorandum 94-1(b). 2nd ed. L.L. Thursone Psychometric Laboratory, University of North Carolina. Chapel Hill, N.C.

Errors in identification using natural markings: rates, sources, and effects on capture–recapture estimates of abundance

Peter T. Stevick, Per J. Palsbøll, Tim D. Smith, Mark V. Bravington, and Philip S. Hammond

Abstract: The results of a double-marking experiment using natural markings and microsatellite genetic markers to identify humpback whales (*Megaptera novaeangliae*) confirm that natural markings are a reliable means of identifying individuals on a large scale. Of 1410 instances of double tagging, there were 414 resightings. No false positive and 14 false negative errors were identified. The rate of error increased with decreasing photographic quality; no errors were observed among photographs of the highest quality rating, whereas an error rate of 0.125 was identified in sightings for which only part of the area used for identification was visible. There was also a weaker relationship between error rate and the distinctiveness of markings, which may result from non-independence in coding for image quality and distinctiveness. A correction is developed for the Petersen two-sample abundance estimator to account for false negative errors in identification, and a parametric bootstrap procedure for estimation of variance is also developed. In application to abundance estimates from the North Atlantic, the correction reduces the bias in estimates made using poorer quality photographs to a negligible level while maintaining comparable precision.

Résumé : Une expérience de marquage double à l'aide de marques corporelles et de marqueurs génétiques microsatellites confirme que l'utilisation des marques corporelles est une méthode fiable d'identification à grande échelle des individus du Rorqual à bosse (*Megaptera novaeangliae*). Des 1410 événements de double marquage, 414 impliquaient des animaux vus antérieurement. Aucun résultat faussement positif ou faussement négatif n'a été décelé. Le taux d'erreur augmentait avec la mauvaise qualité des photographies; aucune erreur ne s'est produite à l'utilisation des photographies de haute qualité et le taux d'erreur était de 0,125 lorsque seulement une partie de la surface de l'animal utilisée pour l'identification était visible. Il y avait aussi une relation plus faible entre le taux d'erreur et le caractère distinctif des marques corporelles, qui peut provenir du manque d'indépendance entre le codage de la qualité de l'image et le caractère distinctif de l'animal. On trouvera ici une correction de l'estimateur d'abondance à deux échantillons de Petersen pour tenir compte des résultats faussement négatifs dans l'identification, ainsi qu'une procédure bootstrap paramétrique pour estimer la variance. Appliquée à des estimations d'abondance provenant de l'Atlantique nord, la correction réduit à un niveau négligeable l'erreur due à l'utilisation de photographies de qualité inférieure dans les estimés, tout en maintenant un niveau de précision similaire.

[Traduit par la Rédaction]

Introduction

Recognition of individual animals from natural markings is an important tool for the study of animal populations and is widely applied to diverse taxa (e.g., Miththapala et al. 1989; Sheldon and Bradley 1989; Bretagnolle et al. 1994). It is common in studies of marine mammals, particularly cetaceans, with application to most of the mysticetes and numerous odontocetes (for a review of methods, see Hammond et al. 1990) and is also applied to pinnipeds (Forcada and Aguilar

Received January 15, 2001. Accepted June 18, 2001. Published on the NRC Research Press Web site at http://cjfas.nrc.ca on August 30, 2001.
J16179

P.T. Stevick.[1] Sea Mammal Research Unit, Gatty Marine Laboratory, University of St. Andrews, St. Andrews, Fife KY16 8LB, Scotland, U.K., and College of the Atlantic, Bar Harbor, ME 04609, U.S.A.
P.J. Palsbøll.[2] School of Biological Sciences, University of Wales, Deiniol Road, Bangor, Gwynedd LL57 2UW, U.K.
T.D. Smith. NOAA National Marine Fisheries Service, Northeast Fisheries Science Center, 166 Water St., Woods Hole, MA 02543, U.S.A.
M.V. Bravington. 7 Holton Terrace, Halesworth, Suffolk, U.K.
P.S. Hammond. Sea Mammal Research Unit, Gatty Marine Laboratory, University of St. Andrews, St. Andrews, Fife KY16 8LB, Scotland, U.K.

[1] Corresponding author (e-mail: pts2@st-andrews.ac.uk).

[2] Present address: Ecosystem Sciences Division – ESPM, University of California Berkeley, 151 Hilgard Hall #3110, Berkeley, CA 94720-3110, U.S.A.

DOI: 10.1139/cjfas-58-9-1861

2000) and sirenians (Reid et al. 1991). Widespread application of this technique to cetaceans has dramatically increased our understanding of population parameters, movement, behaviour, abundance, and structure in many whale populations (e.g., Ford et al. 1994; Smith et al. 1999; Wilson et al. 1999). Capture–recapture studies using naturally marked animals are appropriate in situations where capture is difficult or where there is concern about disturbance of the study population (e.g., Forcada and Aguilar 2000) and thus may be one of the few feasible methods of estimating abundance for some endangered species. Further, for many cetacean species, capture–recapture estimates using naturally marked individuals may be considerably more precise than those typically obtained from line surveys (Hiby and Hammond 1989), the other primary method available.

The likelihood of errors in individual identification has been recognised since the early development of the technique (Bateson 1977; Payne et al. 1983). Incorrect identification may involve falsely identifying two sightings of different individuals as the same (a false positive error) or two sightings of the same individual as different (a false negative error). Errors may be the result of poor quality photographs or observation conditions (Bateson 1977; Agler 1992; Friday et al. 2000), the lack of distinctiveness of the individual markings (Pennycuick and Rudnai 1970; Payne et al. 1983; Friday et al. 2000), or the lack of stability in markings over time (Sheldon and Bradley 1989; Carlson et al. 1990; Bretagnolle et al. 1994). While the problem of identification errors has long been recognised, it has proven fairly intractable; few data on the causes or extent of identification error have been reported.

The use of individual identification for capture–recapture estimates of abundance is based on several assumptions, which have been discussed extensively elsewhere (Seber 1982; Hammond 1986; Pollock et al. 1990). Among them are the assumptions that no animals lose their marks during the study and that all marked animals are reported on recovery. Tag loss and non-reporting have the potential to cause severe bias in parameter estimates (Seber and Felten 1980; Arnason and Mills 1981; Pollock et al. 1991). Other model assumptions influence the appropriate choice of estimator for a particular study but are not relevant to identification error.

Identification errors are in some respects similar to tag loss, though not equivalent. In tag loss, a captured individual is recorded as unmarked when it should be marked. This is irreversible and is a cumulative process, with increasing total loss and often increasing rate of loss with time (Stobo and Horne 1994; Diefenbach and Alt 1998). Visible features can change over time (for example see Carlson et al. 1990; Bretagnolle et al. 1994) and changes that rendered an individual unrecognisable would be the equivalent of tag loss. With most identification errors, however, the individuals remain marked but the marks are not recognised. This is more similar to non-reporting than to tag loss. Previous studies of non-reporting, however, have emphasised reporting rates by hunters, anglers, or commercial fishermen, so the marked animals are removed from the population and are not available for further recapture (Pollock et al. 1991; Stobo and Horne 1994). In errors of identification of naturally marked animals, however, an individual remains in the population and is incorrectly identified as two nominal individuals. The problem of overlooked or unrecognised recaptures of animals that remain in the population and may be subsequently recaptured has received little attention.

Double-marking experiments have been used to estimate retention rates (Stobo and Horne 1994; Diefenbach and Alt 1998) and rates of non-reporting (Pollock et al. 1991; Stobo and Horne 1994) of artificial tags. However, double marking has rarely been applied to studies of natural markings. Previous attempts at double-marking experiments with cetaceans have used natural markings on two areas of the body, one of which is generally less variable than the primary region, and have had limited sample sizes available (Payne et al. 1983; Gowans and Whitehead 2001). Recent advances in genetic techniques, notably the use of microsatellite loci, have made it possible to identify individual animals on a large scale on the basis of genetic markers (Palsbøll et al. 1997*a*). Thus the use of genetic and natural markings in the same population provides two independent sources of identification and an opportunity to examine identification errors.

We report here on the results from a large-scale double-marking experiment in which humpback whales (*Megaptera novaeangliae*) were identified by both natural and genetic markers. This is the first extensive double-marking experiment on a cetacean species. It is also the first large-scale test of errors in individual identification by natural markings for any species. We show that natural markings may be successfully used to identify large numbers of individuals over large spatial scales. We also develop a correction for the two-sample Petersen estimator to account for errors in identification and demonstrate that this correction reduces or eliminates bias resulting from resighting errors.

Methods

Data collection

Data reported here were collected as part of the Years of the North Atlantic Humpback whale project (YoNAH), an international collaborative effort to study the population biology of the humpback whale throughout its range in the North Atlantic Ocean (Smith et al. 1999).

Individual identification photographs were taken of the pigmentation pattern and scars on the ventral side and contours in the trailing edge of the tail flukes (Katona et al. 1979). Protocols for handling of photographs and photographic identification are detailed in Katona and Beard (1990) and Smith et al. (1999). Because the quality of the photograph may influence recognition of individual whales, all photographs were given a quality designation based on the clarity and contrast of the image and the angle of the fluke to the camera. Quality codes range from excellent (1) to acceptable (3), with category 3 images further subdivided into 3+ and 3– based on the extent to which information was obscured by image quality. Quality 4 refers to photographs showing only the right or left sides of the fluke (designated as half flukes) or images showing less than about 20% of the fluke area (designated as partial flukes) irrespective of other photographic quality considerations. This rating reflects the difficulty in re-identifying animals based on only part of the tail being visible.

Distinctiveness of the individual markings may also influence recognition. Each nominal individual was therefore given a distinctiveness rating ranging from very distinctive (1) to poorly marked (3) based on the colour pattern, scarring, and serrations in the trailing edge. Details of the coding process and definitions of the coding criteria are presented by Friday et al. (2000). Additionally, because right and left halves could not be matched to one another, including both in the same sample will artificially decrease recaptures. Therefore, abundance estimates based on samples that included half flukes were calculated separately excluding right halves and excluding left halves.

Skin biopsy samples were collected for genetic analysis as described by Smith et al. (1999). Photographs and biopsy samples were obtained from the same individuals whenever possible. Total-cell DNA was extracted using standard protocols (Maniatis et al. 1982). Genotype was determined at one trinucleotide and five tetranucleotide nuclear microsatellite loci as described by Palsbøll et al. (1997*b*).

Location of errors

Genetic determination and photographic comparison were conducted independently, and the results were not compared prior to this analysis. Identifications resulting from natural markings and from genotype were entered into a relational database along with associated data on sampling location and time. When discrepancies between identifications made by genetic and photographic means were located, the photographs were re-examined to determine whether an error occurred. Discrepancies could result from either false positive or false negative errors in either photographic or genetic identification. Errors may be made during comparison between sightings within a sampling period as well as between samples.

Genetic errors relevant to this sample are discussed elsewhere (Palsbøll et al. 1997*a*). False positive genetic errors were shown to be statistically improbable in a sample of this size. False negative genetic errors were observed but at such a low rate that they are unlikely to influence these results and are not considered further here.

Discrepancies were not necessarily the result of errors in either identification technique but could reflect improper association of photographs and genetic samples. Where discrepancies existed but identification was unambiguous, these discrepancies were designated as non-matching errors. In these cases, the photographs and biopsy samples from one of the sightings were incorrectly associated with one another either in the field or during some stage of handling or analysis.

Correction of abundance estimates

A modification was developed for the standard two-sample Petersen mark–recapture abundance estimator to account for false negative errors in identification. Matching protocols make false positives highly unlikely, and none was observed (see below). We use this modification to examine the effects of identification error on abundance estimates.

There are numerous estimators available for estimation of abundance from capture–recapture results. We use the Petersen estimator, particularly the Chapman's modification (Seber 1982), because this method has previously been applied to results from this study, which provides a comparison with our results. Additionally, in this simple model, it is relatively easy to conceptualise the effect of identification errors.

The Petersen estimator is formulated as

$$\hat{N} = \frac{n_1 n_2}{m_2}$$

where n_1 is the number of individuals identified in sample 1, n_2 is the number of individuals identified in sample 2, and m_2 is the number of individuals identified during both samples.

In this model, individual animals are only counted once within each sampling period; multiple captures of the same individual within a sample are treated as a single identification. Therefore, within a sample i, errors that result in failure to match animals identified at different times will erroneously increase the number of individuals n_i, which is identified from the overall number of identification events s_i. This occurs because a single individual is erroneously treated as two individuals. To account for this, the true value of the number of individuals identified in sample i, n'_i, is estimated as follows.

Let s_i be the number of identification events during sample i, n_i is the observed number of unique individuals in sample i (therefore, $s_i - n_i$ is the observed number of resightings during sample i), a_i is the true number of resightings in sample i, and e_i is the false negative error rate for sample i.

Then

$$s_i - n_i = a_i(1 - e_i)$$

and

$$\hat{a}_i = \frac{s_i - n_i}{1 - e_i}$$

Therefore, an estimate of the true number of individuals identified in sample i is

$$\begin{aligned} n'_i &= s_i - \hat{a}_i \\ &= \frac{s_i(1 - e_i) - s_i + n_i}{1 - e_i} \\ &= \frac{n_i - e_i s_i}{1 - e_i} \end{aligned}$$

When matching between samples, failure to match individuals present in both samples will erroneously reduce the number of recaptures. The observed number of recaptures, m_2, needs to be corrected by the false negative error rate for matching between samples individuals, e_m.

In addition, there is a small chance that a false negative match within a sample could coincide with a false negative match between samples. In this case, a further modification is needed because an identification missed within a sample will not influence the number of recaptures. The corrected m_2 must therefore be reduced by the sum of the proportional errors in each sample, $(n'_i - n_i)/n_i$. Note that this secondary correction ignores the unlikely possibility that the same error occurs in both samples, and it will normally be at least one order of magnitude less than the primary correction.

The true number of recaptures between samples is thus estimated as

$$m'_2 = \left(\frac{m_2}{1 - e_m}\right)\left(1 - \sum_{i=1}^{2} \frac{n'_i - n_i}{n_i}\right)$$

The modified Petersen estimator is then

$$\hat{N}' = \frac{n'_1 n'_2}{m'_2}$$

from which Chapman's modification (Seber 1982) can be calculated as

$$\hat{N}' = \left(\frac{(n'_1 + 1)(n'_2 + 1)}{m'_2 + 1}\right) - 1$$

Photographic error rates are related to image quality. Therefore the false negative error rate for sample i, e_i, is estimated, stratified by image quality, as

$$\hat{e}_i = \sum_{j=1}^{c} p_{ij} f_j$$

where c is the number of photographic quality classes, f_j is the false negative error rate for photographic quality class j, and p_{ij} is the proportion of identification events in sample i, s_i, represented by photographs of photographic quality class j. Some sighting events were represented by multiple photographs; however, comparison was conducted using the best of these and so error rates were calculated using the best quality photograph from each sighting event.

The error rate applicable to recaptures between samples, e_m, is a function of the number of individuals, n_i, not the number of identification events. It is thus estimated as

$$\hat{e}_m = \sum_{j=1}^{c} \frac{n_{1j} + n_{2j}}{n_1 + n_2} f_j$$

Estimation of variance and confidence intervals

No closed form is available for estimating the distribution of abundance estimates that incorporates all of these bias corrections and accounts for the uncertainty in error rate estimates. Thus, variance of the modified estimator was estimated by resampling using the parametric bootstrapping procedure presented in Appendix A. The standard error (SE) was calculated from 1000 estimated values of N', and the 95% confidence interval was estimated using the bias-corrected percentile interval method (Efron and Tibshirani 1993).

This bootstrap procedure is statistically and computationally complex and thus may not be applicable in all circumstances. It is possible to use n_1', n_2', and m_2' to estimate variance using the analytical formula for Chapman's estimator (Seber 1982) as

$$\hat{\mathrm{var}}\ \hat{N}' = \frac{(n_1' + 1)(n_2' + 1)(n_1' - m_2')(n_2' - m_2')}{(m_2' + 1)^2 (m_2' + 2)}$$

This method does not account for either the variance of the error rate estimates or for sampling heterogeneity, however, and thus will underestimate variance.

Results

During 1992 and 1993, 4207 photographic samples and 2326 biopsy samples were collected. Following Smith et al. (1999), identifications of the same individual on separate days were considered separate identification events. There were 1410 instances of double tagging. Of these, there were 414 cases in which double-tagged animals were re-identified by one method or the other (or both) during another identification event where double tagging occurred so that identification by the two methods could be compared. This resighting proportion is not significantly different than that for photographs alone ($\chi^2 = 0.64$, $p = 0.42$).

In most of these resightings, identifications by natural markings and genetics both matched between sightings. However, a total of 88 discrepancies between photographic and genetic identifications were found: 37 in which the photographic identifications were the same and the genetic identifications different, and 51 in which the genetic identifications were the same and the photographic identifications different.

Errors identified

By far the largest source of discrepancies was the failure to link photographs and biopsies correctly; 74 such non-matching errors were identified. These could have resulted in the field from confusion over the identity of the whales sampled within a group, difficulty in interpreting field notes during initial data analysis or mislabelling of biopsy samples or photographs, handling errors in the lab, or even typographical errors. The mean group sizes from which non-matching errors were identified (3.07 whales per group, median 3) were larger than those of double-tagged animals from which no discrepancies were identified (2.74 whales per group, median 2). Because these data were not normally distributed, a non-parametric test was used. The distributions were significantly different (Mann–Whitney $U = 53\,089$, $p = 0.008$), suggesting that confusion of animals is more likely to occur in larger groups of animals.

A total of 14 errors was identified. All were false negatives; no false positive errors were identified. Of these errors, five were based on sightings represented only by half or partial fluke photographs. Of the remaining nine, four cases were based on sightings with photographs that, while meeting minimum quality standards, were exceedingly difficult to match to one another because of differences in angle, contrast, clarity, or portion of fluke visible. The information in these photographs was consistent with them coming from the same individual, and they are recorded here as photographic errors. Some ambiguity remains in the identifications, however, and the possibility that some are non-matching errors cannot be entirely ruled out.

Table 1 shows the relationship between errors identified and the image quality and individual distinctiveness ratings. The qualities of the photographs from both sightings are likely to contribute to the probability of errors. Preliminary investigation of these data, however, indicated that the role of the photographic quality from the sighting with better quality was minor and was not analysed further.

While the number of observations in each cell is small, error rates increase steadily with decreasing image quality. They range from no errors identified for quality 1 photographs to over 12% for cases in which at least one sighting is represented only by half or partial photographs. The range of error rates varies less between distinctiveness categories than between photographic qualities. Within distinctiveness categories, a relationship with photographic quality is apparent in spite of the small sample sizes in each cell.

Because error rates are related to image quality, for the rates determined in the double-marking experiment to be an accurate representation of overall error rates in the sample, the double-tagged individuals should be representative of the distribution of image quality in the sample as a whole. The experimental subset is nearly 10% of the overall photographic sample, and most photograph quality and distinctiveness classes are also very close to 10% of those overall except in cases where sample sizes are small.

Estimates of abundance

Four estimates of humpback whale abundance in the North Atlantic Ocean were recently presented by Smith et al. (1999). These were based on one winter sample from the low-latitude breeding range and one summer sample from the high-latitude feeding range to minimise heterogeneity of capture probability between samples. They were not corrected for identification error. However, they were restricted to photographs of quality 3+ or better to minimise error related bias. Table 2

Table 1. Distribution of error rates by photographic quality and distinctiveness categories.

Distinctiveness quality	Photographic quality 1	2	3+	3–	4	Total
1	0	0	0	0	1.00	0.028
	(0, 23)	(0, 39)	(0, 7)	(0, 1)	(2, 2)	(2, 72)
2	0	0.033	0.026	0.125	0.083	0.030
	(0, 66)	(4, 121)	(2, 77)	(1, 8)	(2, 24)	(9, 296)
3	0	0	0.222	0	0.091	0.073
	(0, 8)	(0, 12)	(2, 9)	(0, 1)	(1, 11)	(3, 41)
Total	0	0.023	0.043	0.091	0.125	0.034
	(0, 97)	(4, 172)	(4, 94)	(1, 11)	(5, 40)	(14, 414)

Note: Figures in parentheses are numbers of errors observed and the sample size for each category, respectively. Assuming that errors are a function of the poorest image in the comparison rather than both, this table presents the distribution of only the sighting with the poorest quality images. Where distinctiveness ratings disagreed, images were compared to determine the correct rating. Totals for photographic quality columns do not necessarily equal the sums of those columns as some individuals were given a distinctiveness code of "Unknown".

Table 2. Comparison of corrected and uncorrected estimates of abundance of humpback whales in the North Atlantic Ocean during 1992 and 1993.

Sample	s	n	e	n'
Winter 1992	683	629	0.0248	627.6
Summer 1992	1024	787	0.0224	781.6
Winter 1993	623	582	0.0257	580.9
Summer 1993	1293	937	0.0200	929.7

Estimate	n'_1	n'_2	m_2	e_m	m'_2
W_{92}–S_{92}	627.6	781.6	39	0.0219	40.2
W_{93}–S_{93}	580.9	929.7	52	0.0209	53.6
W_{92}–S_{93}	627.6	929.7	48	0.0206	49.5
W_{93}–S_{92}	580.9	781.6	48	0.0224	49.5

Not error corrected

		Analytical		Bootstrap	
Estimate	Abundance	SE	CV	SE	CV
W_{92}–S_{92}	12 410	1828	0.147	2095	0.159
W_{93}–S_{93}	10 320	1300	0.126	1454	0.134
W_{92}–S_{93}	12 060	1595	0.132	1871	0.146
W_{93}–S_{92}	9 370	1229	0.131	1418	0.143

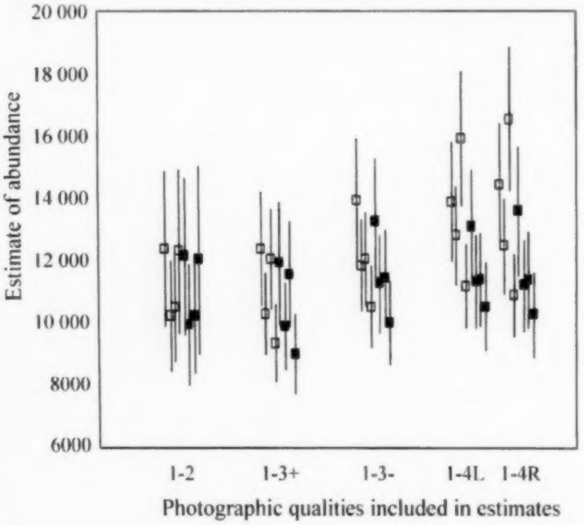
Error Corrected

		Analytical		Bootstrap		
Estimate	Abundance	SE	CV	SE	CV	Bias
W_{92}–S_{92}	11 930	1729	0.145	1961	0.159	1.040
W_{93}–S_{93}	9 910	1229	0.124	1361	0.134	1.041
W_{92}–S_{93}	11 580	1506	0.130	1676	0.143	1.041
W_{93}–S_{92}	9 160	1161	0.127	1268	0.138	1.040

Note: Uncorrected estimates are those presented by Smith et al. (1999). Both corrected and uncorrected estimates are made with Chapman's modification and are limited to images of qualities of 3+ or better to be comparable with Smith et al. (1999). Analytical variances are calculated as given in Seber (1982). Corrected estimates and bootstrap standard errors (SE) are calculated using the methods presented in the text. For each sample, the number of sampling events (s), the observed number of individuals identified (n), the calculated error rate (e), and the estimated number of individuals actually identified (n') are presented. Each estimate is based on one winter and one summer sample: W_{92}, winter 1992; W_{93}, winter 1993; S_{92}, summer 1992; S_{93}, summer 1993. For each, the observed number of recaptures (m_2), the calculated error rate (e_m), and the estimated actual number of recaptures (m'_2) are presented. The proportional bias (uncorrected/corrected) is given for each estimate. CV, coefficient of variation.

presents these four estimates along with the calculated error rates, estimates, and proportional bias (uncorrected/corrected) for corrected estimates using the same photographic quality selection for comparison. Because there were no false positives identified in the double-marking experiment, all biases in abundance estimates are positive. The proportional biases are highly consistent among the four estimates, ranging only from 1.040 to 1.041. This is true in spite of considerable

Fig. 1. Change in four winter–summer estimates of abundance for North Atlantic humpback whales from data collected during 1992 and 1993 (Smith et al. 1999) with inclusion of photographs of decreasing quality (±SE). Uncorrected estimates (open squares) were made with the standard Chapman's estimator, whereas corrected estimates (solid squares) incorporate the correction for identification errors presented in the text. Photographic quality categories used are those presented by Friday et al. (2000). Category 4 includes photographs coded as half or partial. Within category 4, two estimates are made for each combination of winter–summer samples, one excluding right halves (4L) and another excluding left halves (4R).



variability in within-sample resighting rates, between-sample recapture rates, and image quality between samples and estimates. The precision of corrected and uncorrected estimates is comparable if both are determined using the same method. Use of the analytical formula underestimated variance by between 6 and 11% in comparison with the corresponding bootstrapped estimates in the examples presented here. This level of bias was similar for corrected and uncorrected estimates.

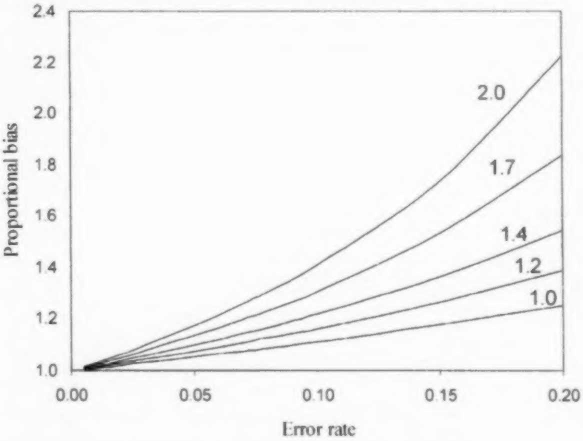
Errors lead to substantial increases in uncorrected abundance estimates, as photographs of progressively poorer quality are included in the sample. Application of the correction to these estimates substantially reduces the variability in estimates made with different photographic qualities, effectively eliminating this bias (Fig. 1).

Sensitivity analysis demonstrated that the error correction is not influenced by absolute abundance or recapture rates provided that the error rates and within-sample resighting rates remain the same. Biases are sensitive, however, to variability in resighting rates (Fig. 2). Higher resighting rates inflate bias but not appreciably at the error and resighting rates reported here.

Discussion

Comparison of results from photographic and genetic identification confirms that natural markings provide a reliable means of identifying individuals on a large scale. While errors in identification will cause inflation of abundance estimates if not taken into account, the error rates for photographic identification reported here are low relative to other sources of variability in recapture rates. Further, knowledge of the error rates allows for a correction to be applied.

Fig. 2. Effects of error rates on proportional bias in estimates of abundance. Curves represent different resighting rates (the number of sighting events in the sample/the number of unique individuals identified in that sample). Thus a resighting rate of 1 indicates that each individual was sighted only once during the sample, while a resighting rate of 2 indicates a mean of two resightings per identified individual within the sample. Results are insensitive to variability in between-sample recapture rate. Approximate resighting rates from this project are 1.1 for breeding-ground samples and 1.3 for feeding-ground samples.



While the error rate is low, the relatively large number of non-matching errors suggests that considerable care should be given to ensuring that samples and photographs are linked correctly to sighting records, particularly where group sizes are large. Fortunately, non-matching errors simply indicate a failure to correctly link two types of data collected from an individual. Thus they do not reflect upon the reliability of either data set if it is used independently, and they have no impact on capture–recapture estimates of abundance made by any single identification method. Care is especially important, however, when information gained from genetics is associated with other information associated with photographs, or if identifications by the two methods are combined for a single analysis.

False positive errors

It has been observed that false positive errors produce large biases in estimates of population parameters and that they are especially problematic in large collections where they may cause substantial biases in two sample estimators even at low rates (Gunnlaugsson and Sigurjónsson 1990). Schwartz and Stobo (1999) examined the influence of false positive errors on open population models. Errors were caused by misreading of tags, which resulted in high false positive rates (0.10, SE 0.015) but no false negatives. Large biases in parameter estimates were observed, with capture probabilities overestimated by between 4% and 34%. Thus it is reassuring to find no evidence of false positive error in this experiment.

However, risk aversion to false positives inevitably increases the number of false negatives, and the balance between these factors has not been investigated in practice. The theoretical basis for concern over the influence of false positive errors assumes that these errors are equally likely in each comparison made (Gunnlaugsson and Sigurjónsson 1990). In practice, the likelihood of a false positive error is a function of the similarity of the markings of the individuals compared. Thus, if the information content on which identification is based is high, the number of comparisons at risk for false positive errors is correspondingly low.

The lack of false positive errors in photographic identification shown here probably reflects the caution displayed in matching protocols. Previous experimental results have suggested that false positives are likely in photographic identification. Carlson et al. (1990) reported a range of false positive errors in identification of humpback whales from 0.0053 to 0.0951, while a similar experiment on fin whales, where markings are more difficult to identify, reported a false positive rate ranging from 0.015 to 0.187 (Agler 1992). In both studies, error rates were related to the experience of the personnel undertaking the matching. These experiments, however, examined the decisions of a single individual. In practice, matching protocols are generally risk averse to false positives. Identifications are made by experienced individuals, use restrictive criteria, and (or) are confirmed by at least two individuals, substantially reducing the probability of errors (IWC 1990). In the results presented here, all identifications were checked by at least two experienced staff, and cases where information was inadequate or where consensus could not be reached were resolved in favor of not matching.

False negative errors

False negative errors are well known to occur in studies of natural markings (Bateson 1977; Carlson et al. 1990; Agler 1992). Carlson et al. (1990) reported false negative error rates in recognition of humpback whales among experimental subjects ranging from 0.0075 to 0.0233. Agler (1992) found a false negative error rate of 0.107 for fin whales among the most experienced personnel. The relative obscurity of markings in fin whales compared with humpbacks makes it difficult to compare results between species. Forcada and Aguilar (2000) identified no errors in comparison of multiple photographic sightings of 14 individual monk seals also marked with artificial tags, yet they showed considerable bias in estimates made with the poorest quality photographs, indicating the presence of such errors in their data. These experiments were conducted on small sets of photographs, although it has been suggested that the rate of errors is a function of fatigue and will therefore increase with increasing catalogue size (Gunnlaugsson and Sigurjónsson 1990).

Both photographic quality and distinctiveness appear to be related to error rates, and both may play some role in the making of errors. Errors related to photo quality and distinctiveness violate assumptions of capture–recapture estimation in different ways (Hammond 1986). Errors related to the distinctiveness of markings influence the individual probability of identification for an animal, leading to heterogeneity of sampling probabilities across samples. This is not the case for errors related to photographic quality where it is reasonable to assume that the quality of a photograph is independent of the animal it represents. Photographic quality and distinctiveness are difficult to determine independently. It has been shown that coders in this study gave poor quality photographs consistently poorer distinctiveness codes (Friday 1997; Friday et al. 2000). On the other hand, coders appeared to code photographic quality independent of the distinctiveness of the animal.

In this study, no errors were identified among those sightings with the best quality photographs regardless of the distinctiveness rating of the individual. Among whales with the highest distinctiveness rating, errors were identified only among half and partial photographs. However, the strong correlation between image quality and error rate, with a virtual doubling of error rate with each sequential decline in quality designation, and the less evident trend between error rates and distinctiveness ratings indicate that image quality is a more important determinant of error rate than is distinctiveness. Further, given the lack of independence between photographic quality and distinctiveness coding, the increase in error rate with decreasing distinctiveness rating observed here may indirectly be the result of image quality.

Pattern change has been implicated in error rates (Carlson et al. 1990; Wilson et al. 1999; Gowans and Whitehead 2001); however, none of the errors identified here was the result of pattern change. The time period of this study is short relative to the life expectancy of humpback whales, thus pattern change may be less evident than in longer term studies and may have different implications, therefore, for use of multiple recapture models. However, humpback whale markings are remarkably stable in most instances; changes in ventral fluke patterns of humpback whales substantial enough to compromise identification are rare and occur most often in young animals (Carlson et al. 1990; Blackmer et al. 2000).

Samples represented only by photographs coded as right or left halves or as partial accounted for five of the 14 errors identified, giving an error rate of over 12%. This indicates that photographs that do not show all of the fluke area are subject to higher rates of identification error, even when a considerable amount of information is present in the area photographed.

Influence on estimates of abundance

Our results show that errors in identification positively bias abundance estimates. Even if not accounted for, however, bias resulting from identification errors proved small compared with the variability between estimates and the precision of the estimates. This bias can be substantially reduced but not eliminated by removing the poorest quality photographs. This is normally the approach adopted in capture–recapture studies using natural markings. The four cases of errors from images rated as acceptable, but where matching was none-the-less ambiguous, indicate that not all errors can be eliminated where photographic quality is poor, even with nearly perfect photographic comparison. These errors could be eliminated, or nearly so, by implementing more stringent photo quality standards. However, this would substantially reduce the sample size and thus result in loss of precision.

Knowledge of error rates specific to image quality allows corrections to be made that virtually eliminate the bias resulting from identification errors. This allows inclusion of more samples, improving precision of abundance estimates while introducing negligible bias.

Acknowledgements

This work was conducted as part of the YoNAH project and would have been impossible without the support and hard work of Judy Allen, Phil Clapham, Steve Katona, Finn Larsen, Jon Lien, David Mattila, Johann Sigurjónsson, and Nils Øien. The field and laboratory effort involved in collecting and processing these samples was staggering and involved dozens of individuals from many institutions. Photographic comparison was conducted at College of the Atlantic. Special thanks are due to Tom Fernald and Nancy Stevick for their extensive work on photographic analysis and quality coding. The genetics laboratory work was conducted at the Department of Evolutionary Biology (Zoological Institute, University of Copenhagen) and School of Biological Sciences (University of Wales, Bangor) with assistance from Martine Bérubé, Pernille Feddersen, Hanne Jørgensen, and Anja Holm Larsen. Our understanding of the identification, sources, and effects of errors was enhanced by discussions with Steve Buckland, Dan DenDanto, Nancy Friday, Shannon Gowans, and Ken Pollock. Critical reading of the manuscript by Steve Buckland, John Calambokidis, Rob Harcourt, and two anonymous reviewers improved the analysis and presentation. Funding for this analysis was provided by the W.E. Anderson Trust and by the National Oceanic and Atmospheric Administration through National Marine Fisheries Service contact 40ENNF900103 to PTS. The views expressed herein are those of the authors and do not reflect the views of NOAA or any of its subagencies.

References

Agler, B.A. 1992. Testing the reliability of photographic identification of individual fin whales (*Balaenoptera physalus*). Rep. Int. Whal. Comm. **42**: 731–737.

Arnason, A.N., and Mills, K.H. 1981. Bias and loss of precision due to tag loss in Jolly-Seber estimates for mark–recapture experiments. Can. J. Fish. Aquat. Sci. **38**: 1077–1095.

Bateson, P.P.G. 1977. Testing an observer's ability to identify individual animals. Anim. Behav. **25**: 247–248.

Blackmer, A.L., Anderson, S.K., and Weinrich, M.T. 2000. Temporal variability in features used to photo-identify humpback whales (*Megaptera novaeangliae*). Mar. Mamm. Sci. **16**: 338–354.

Bretagnolle, V., Thibault, J., and Dominici, J. 1994. Field identification of individual ospreys using head marking pattern. J. Wildl. Manag. **58**: 175–178.

Carlson, C.A., Mayo, C.C., and Whitehead, H. 1990. Changes in the ventral fluke pattern of the humpback whale (*Megaptera novaeangliae*), and its effect on matching: evaluation of its significance to photo-identification research. Rep. Int. Whal. Comm. Spec. Issue, **12**: 105–111.

Diefenbach, D.R., and Alt, G.L. 1998. Modelling and evaluation of ear tag loss in black bears. J. Wildl. Manag. **62**: 1292–1300.

Efron, B., and Tibshirani, R.J. 1993. An introduction to the bootstrap. Chapman and Hall, New York.

Forcada, J., and Aguilar, A. 2000. Use of photographic identification in capture–recapture studies of Mediterranean monk seals. Mar. Mamm. Sci. **16**: 767–793.

Ford, J.K.B., Ellis, G., and Balcomb, K.C. 1994. Killer whales: the natural history and geneology of *Orcinus orca* in the waters of British Columbia and Washington State. University of British Columbia Press, Vancouver, B.C.

Friday, N. 1997. Evaluating photographic capture–recapture estimates of abundance of North Atlantic humpback whales. Ph.D. thesis, Oceanography, University of Rhode Island. Bell and Howell Information and Learning No. 9805232.

Friday, N., Smith, T.D., Stevick, P.T., and Allen, J. 2000. Measurement of photographic quality and animal distinctiveness for the photographic identification of humpback whales. Mar. Mamm. Sci. **16**: 355–374.

Gowans, S., and Whitehead, H. 2001. Photographic identification of northern bottlenose whales (*Hyperoodon ampullatus*): sources of heterogeneity from natural marks. Mar. Mamm. Sci. **17**: 76–93.

Gunnlaugsson, T., and Sigurjónsson, J. 1990. A note on the problem of false positives in the use of natural marking data for abundance estimation. Rep. Int. Whal. Comm. Spec. Issue, **12**: 143–145.

Hammond, P.S. 1986. Estimating the size of naturally marked whale populations using capture–recapture techniques. Rep. Int. Whal. Comm. Spec. Issue, **8**: 253–282.

Hammond, P.S., Mizroch, S.A., and Donovan, G.P. 1990. Individual recognition of cetaceans: use of photo-identification and other techniques to estimate population parameters. Rep. Int. Whal. Comm. Spec. Issue **12**.

Hiby, A.R., and Hammond, P.S. 1989. Survey techniques for estimating abundance of cetaceans. Rept. Int. Whal. Comm. Spec. Issue, **11**: 47–80.

IWC (International Whaling Commission). 1990. Report of the workshop on individual recognition and the estimation of cetacean population parameters. Rep. Int. Whal. Comm. Spec. Issue, **12**: 3–17.

Katona, S.K., and Beard, J.A. 1990. Population size, migrations and feeding aggregations of the humpback whale (*Megaptera novaeangliae*) in the western North Atlantic Ocean. Rep. Int. Whal. Comm. Spec. Issue, **12**: 295–305.

Katona, S.K., Baxter, B., Brazier, O., Kraus, S., Perkins, J., and Whitehead, H. 1979. Identification of humpback whales by fluke photographs. *In* The behavior of marine animals. Vol. 3. Cetacea. *Edited by* H.E. Winn and B.L. Olla. Plenum Press, New York. pp. 33–44.

Maniatis, T., Frisch, E.F., and Sambrook, J. 1982. Molecular cloning (a laboratory manual). Cold Spring Harbor Laboratory, Cold Spring Harbor, N.Y.

Miththapala, S., Seidensticker, J., Phillips, L.G., Fernando, S.B.U., and Smallwood, J.A. 1989. Identification of individual leopards (*Panthera pardus kotiya*) using spot pattern variation. J. Zool. (Lond.), **218**: 527–536.

Palsbøll, P.J., Allen, J., Bérubé, M., Clapham, P.J., Feddersen, T.P., Hammond, P.S., Hudson, H. Jorgensen, S. Katona, A. H. Larsen, F. Larsen, J. Lien, D. K. Mattila, J. Sigurjónsson, R.R., Sears, R., Smith, T., Sponer, R., Stevick, P., and Øien, N. 1997*a*. Genetic tagging of humpback whales. Nature (Lond.), **388**: 767–769.

Palsbøll, P.J., Bérubé, M., Larsen, A.H., and Jórgensen, H. 1997*b*. Primers for the amplification of tri- and tetramer microsatellite loci in cetaceans. Mol. Ecol. **6**: 895–897.

Payne, R., Brazier, O., Dorsey, E.M., Perkins, J.S., Rowntree, V.J., and Titus, A. 1983. External features in southern right whales

(*Eubalaena australis*) and their use in identifying individuals. *In* Communication and behavior of whales. *Edited by* R. Payne. Westview Press, Boulder, Co. pp. 371–445.

Pennycuick, C.J., and Rudnai, J. 1970. A method of identifying lions *Panthera leo* with an analysis of the reliability of identification. J. Zool. (Lond.), **218**: 527–536.

Pollock, K.H., Nichols, J.D., Brownie, C., and Hines, J.E. 1990. Statistical inference for capture–recapture experiments. Wildl. Monogr. **107**: 1–97.

Pollock, K.H., Hoenig, J.M., and Jones, C.M. 1991. Estimation of fishing and natural mortality when a tagging study is combined with a creel survey or port sampling. Am. Fish. Soc. Symp. **12**: 423–434.

Reid, J.P., Rathbun, G.B., and Wilcox, J.R. 1991. Distribution patterns of individually identifiable West Indian manatees (*Trichechus manatus*) in Florida. Mar. Mamm. Sci. **7**: 180–190.

Schwartz, C.J., and Stobo, W.T. 1999. Estimation and effects of tag-misread rates in capture–recapture studies. Can. J. Fish. Aquat. Sci. **56**: 551–559.

Seber, G.A.F. 1982. The estimation of animal abundance and related parameters. Charles Griffin and Company, London.

Seber, G.A.F., and Felton, R. 1980. Tag loss and the Peterson mark–recapture experiment. Department of Mathematics Rep. No. 162, University of Aukland, Aukland, N.Z.

Sheldon, S., and Bradley, C. 1989. Identification of individual adders (*Vipera berus*) by their head markings. Herpetol. J. **1**: 392–396.

Smith, T.D., Allen, J., Clapham, P.J., Hammond, P.S., Katona, S., Larsen, F., Lien, J., Mattila, D., Palsbøll, P.J., Sigurjónsson, J., Stevick, P.T., and Øien, N. 1999. An ocean-basin-wide mark–recapture study of the North Atlantic humpback whale (*Megaptera novaeangliae*). Mar. Mamm. Sci. **15**: 1–32.

Stobo, W.T., and Horne, J.K. 1994. Tag loss in grey seals (*Halichoerus grypus*) and potential effects on population estimates. Can. J. Zool. **72**: 555–561.

Wilson, B., Hammond, P.S., and Thompson, P.M. 1999. Estimating size and assessing trends in a coastal bottlenose dolphin population. Ecol. Appl. **9**: 288–300.

Appendix A. Variance estimates and confidence intervals

Estimates of variance and confidence limits for N can be obtained by the parametric bootstrap procedure described below. The bootstrap is designed to be robust against within-sample heterogeneity of capture probability; this might arise, for example, from different whales having different patterns of habitat use and thus different exposure to photograph opportunities. Note that between-sample heterogeneity must be minimal to meet the assumptions of the estimator and thus is not accounted for in the bootstrap.

The basic idea of the bootstrap is to estimate the pattern of heterogeneity based on the quality-1 photographs and then apply the pattern to photographs of all qualities when simulating the data. Information on heterogeneity is provided by the distribution of b_m (the number of whales with m quality-1 photographs). Values of b_1, b_2, etc., can be taken from the raw data, and b_0 can be obtained by subtracting the sum of these from the estimate of total abundance.

In any given season, suppose that the ith whale has an "intrinsic photographability" of λ_i, defined as the expected number of quality-1 photographs. The number of quality-1 photographs of that whale follows a Poisson distribution with a mean λ_i, and for a randomly chosen whale from the whole population, the number of quality-1 photographs follows a compound Poisson distribution:

$$P[\text{number of photographs} = m] \approx (m!)^{-1}\int e^{-\lambda}\lambda^{m} f_{\lambda}(\lambda)\mathrm{d}\lambda,$$

where $f_\lambda(\lambda)$ is the population distribution of λ.

If all photograph probabilities are the same (i.e., the distribution of λ is concentrated at a single point), then the distribution of m across all whales will also be Poisson. This can be studied by a χ^2 test. Table A1 shows that the equal-probability hypothesis is tenable for the breeding-ground data set (where there is no whale that has more than one quality-1 photograph, which is a good fit to a Poisson distribution) but not for the feeding-ground data (χ^2 statistic on groups 0, 1, and 2+ is 87.47, 1 df, $p < 0.0001$), so that estimation of heterogeneity is required. If we were only interested in quality-1 photographs, all that would be necessary would be to resample from the empirical distribution of m. However, because we need to simulate all qualities of photograph, it is necessary to estimate something about the underlying distribution of λ. Clearly, we only have a small number of informative observations (number of whales with 0 quality-1 photos, number of whales with 1 quality-1 photograph, etc.) so we cannot expect to recover a full continuous distribution for λ. Fortunately, all that is needed in practice is an approximate distribution for λ that mimics the data. An efficient way to do this is to assume that λ follows a discrete distri-

Table A1. Number of whales with different numbers of quality-1 photographs from the summer 1992 and winter 1992 data sets.

		Number of whales with 0–3 quality-1 photographs			
		0	1	2	3
Feeding 1992	Observed	12 447	228	14	4
	Expected	12 428	262	2.8	0.02
Breeding 1992	Observed	12 558	135	0	0
	Expected	12 559	134	0.7	0

Note: Abundance is 12 693 (standard Petersen estimate). For Feeding 1992, the maximum number of sightings represented by quality-1 photographs per whale was 3. For Breeding 1992, the maximum number of sightings represented by quality-1 photographs per whale was 1.

bution with support at R prespecified points $\pi_1,\ldots,\pi_R$, and corresponding weights $w_1,\ldots,w_R$ to be estimated, subject to the constraints $w_r \geq 0$, $\Sigma w_r = 1$.

Given a fixed set π, constrained maximum likelihood estimates of w can be found by iterative quadratic programming. Some experimentation is needed to select an appropriate value for π. However, equally good fits to the data can be obtained with many different πs, and the precise choice is irrelevant; in effect, the Poisson variability smears out fine details in the distribution of λ. The iterative quadratic programming estimates of w seem numerically stable as long as R is no larger than the maximum number of quality-1 photographs of any individual whale in the season.

Once we have estimated the extent of heterogeneity for quality-1 photographs, it is easy to work out the distribution of all qualities of photographs, assuming photograph quality is independent of the number of times a whale is photographed. Suppose the ith whale has intrinsic photographability λ at quality 1. The distribution of number of quality-1 photographs of that whale is Poisson with mean λ, and the distribution of number of photographs of any quality for that whale is simply Poisson with mean λ/q_1, where $q_1 = P$[quality of photograph = 1]. This leads to a conceptually simple parametric resampling scheme, as follows.

1. For the ith whale in the population, randomly pick its quality-1 photograph rate π^{i*} from the set π according to the probabilities w.
2. Pick m^{i*}, the total number of photographs of that whale, from the Poisson distribution with mean π^{i*}/q_1.
3. Allocate the m^{i*} photographs to quality category according to a multinomial distribution with probabilities $q_1, q_2, \ldots$.
4. For each photograph that is not of quality-1, decide whether it is correctly identified to the ith whale using a Bernoulli distribution.

Although this procedure is simple, repeating it for all $\hat{N}$ whales is very slow. It is much more efficient to start by deciding how many whales get photographed 0, 1, 2,…, times by sampling from a multinomial distribution with limit $\hat{N}$ and $P[m] = \sum_r w_r \exp\{-\pi_r/q_1\}(\pi_r/q_1)^m/m!$. The whales not photographed at all (the vast majority) can then be ignored, and steps 3–4 above can be applied to each of the remaining whales.

PERSPECTIVES

Using demographic methods to construct Bayesian priors for the intrinsic rate of increase in the Schaefer model and implications for stock rebuilding

M.K. McAllister, E.K. Pikitch, and E.A. Babcock

Abstract: Even though Bayesian methods can provide statistically rigorous assessments of the biological status of fisheries resources, uninformative data (e.g., declining catch rate series with little variation in fishing effort) can produce highly imprecise parameter estimates. This can be counteracted with the use of informative Bayesian prior distributions (priors) for model parameters. We develop priors for the intrinsic rate of increase (r) in the Schaefer surplus production model using demographic methods and illustrate the utility of this with an application to large coastal sharks in the Atlantic. In 1996, a U.S. stock assessment obtained a point estimate for r of 0.26. For such long-lived and low-fecund organisms, this could potentially be too high. Yet it was used to predict that within about 10 years, a 50% reduction in the 1995 catch level should result in >50% chance of increasing the population to the abundance required to produce maximum sustainable yield. In contrast, a Bayesian assessment that used demographic analysis to construct a prior for r with a median of 0.07 and coefficient of variation (CV) of 0.7 indicated that within 30 years, this policy would have only a very small chance of increasing the population to maximum sustainable yield.

Résumé : Bien que les techniques bayésiennes puissent fournir des estimations statistiquement rigoureuses du statut biologique des ressources halieuthiques, des données pauvres en information (e.g., un série de taux de capture associée à un effort de pêche peu variable) peuvent produire des estimations de paramètres très imprécises. Ce problème peut être contrecarré par l'utilisation de distributions bayésiennes a priori (priors) pour les paramètres du modèle. Nous avons développé des distributions a priori pour le taux intrinsèque de croissance (r) dans le modèle de production excédentaire de Schaefer à l'aide de méthodes démographiques et nous illustrons l'utilité de cette approche en l'appliquant aux grands requins côtiers de l'Atlantique. En 1996, une évaluation de stock faite aux États-Unis a donné une estimation ponctuelle de r de 0,26. Pour des organismes à grande longévité et à faible fécondité, cette valeur est potentiellement trop élevée. Néanmoins, elle a servi à prédire que, dans à peu près 10 ans, une réduction de 50% dans le taux de capture de 1995 aurait pour conséquence une probabilité de >50% de faire croître la population suffisamment pour atteindre le niveau de rendement maximal soutenu. En revanche, une estimation de type bayésien qui a utilisé une analyse démographique pour établir une distribution a priori de r avec une médiane de 0,07 et un coefficient de variation de 0,7 indique qu'une telle politique de capture n'aurait, au bout de 30 ans, qu'une très faible probabilité de permettre à la population d'atteindre le rendement maximal soutenu.

[Traduit par la Rédaction]

Introduction

Bayesian estimation has been advocated as a statistically rigorous approach to account for and reduce uncertainties arising from data analysis (Gelman et al. 1995; Punt and Hilborn 1997; McAllister and Kirkwood 1998*a*). However, when these methods are used to model uncertainty, particularly in fisheries stock assessment, model predictions can still be highly imprecise and biased (Adkison and Peterman 1996). This is often because data for individual fish stocks

Received October 11, 2000. Accepted June 13, 2001. Published on the NRC Research Press Web site at http://cjfas.nrc.ca on August 30, 2001.
J16014

M.K. McAllister.[1] Renewable Resources Assessment Group, Department of Environmental Science and Technology, Imperial College, Royal School of Mines Building, Prince Consort Road, London, SW7 2BP, U.K.
E.K. Pikitch and E.A. Babcock. Wildlife Conservation Society, 185 Street and Southern Boulevard, Bronx, NY 10460, U.S.A.

[1] Corresponding author (e-mail: mmmcal@ic.ac.uk).

DOI: 10.1139/cjfas-58-9-1871

are uninformative about key population dynamics model parameters, for example, the maximum population growth rate, r, which can determine the rate of population recovery following severe depletion and a reduction in harvesting (Liermann and Hilborn 1997; Myers et al. 1997).

Estimation uncertainty can be reduced with the application of informative Bayesian prior probability density functions (pdfs) for model parameters (McAllister et al. 1994). Hierarchical modeling methods have recently been developed to help construct these priors (Gelman et al. 1995; Liermann and Hilborn 1997). Myers et al. (1997, 1999), for example, provided a hierarchical modeling approach that was applied by Millar and Meyer (2000) to construct a prior pdf for r that incorporates stock and recruit data sets, estimates of the age at maturity, and the rate of natural mortality in adults. These methods, however, require data sets from several other similar (e.g., conspecific or congeneric) populations. In many instances, for example, for most shark populations, such data sets are not available. We formulate in this paper an alternative approach to constructing a prior for r that uses demographic methods, which do not require data from other populations (Krebs 1985; Begon et al. 1996; Ebert 1999). We illustrate this approach with an application to demographic and catch and catch rate data for large coastal sharks (LCSs) off the U.S. Atlantic coast.

This paper is organized as follows. First, we formulate a demographic approach to constructing a prior distribution for r. Second, we illustrate the utility of this approach with an application to data for LCSs. Bayesian posterior estimates of stock status and potential for recovery to the maximum sustainable yield level (MSYL) are assessed with and without the informative prior for r.

Using demographic analysis to construct a prior probability distribution for the intrinsic rate of increase

Demographic theory provides two conceptually interrelated definitions of the intrinsic rate of natural increase (Krebs 1985, pp. 182–184). The first is the observed rate of increase per individual per unit time, r_o, for a population in its natural environment. The r_o varies continuously from – to + in response to changes in food availability, age distribution, and environmental conditions, among other things. The second is the innate capacity for increase, r_m. This is the maximum per capita rate of increase attained under some particular set of abiotic conditions when the quantities of food, space, and animals in the population are kept to an optimum and other species are entirely excluded from the situation. In other words, this is the maximum population growth rate owing to optimal biological conditions at a given set of abiotic conditions. This quantity is often used as a reference point for comparisons with observed rates of increase in nature. Demographic theory also specifies that under constantly maintained abiotic and optimal biological conditions, a population would reach a stable age structure and increase in numbers according to the differential equation

$$(1) \qquad \frac{\mathrm{d}N}{\mathrm{d}t} = r_m N$$

These formulations parallel analogous ones associated with the age-aggregated logistic population dynamics model. In this model, the intrinsic rate of increase, r_L, is defined as the maximum per capita rate of increase. Thus, r_L and r_m are analogous, except that r_L is realized without any assumptions about age structure as abundance approaches zero, when density-dependent effects that reduce the rate of population growth are assumed to be at a minimum:

$$(2) \qquad \frac{\mathrm{d}N}{\mathrm{d}t} = \lim_{N \to 0} r_L N$$

The realized per capita rate of increase, r_r, in the logistic model (eq. 17) varies with population abundance and is thereby analogous to r_o in demographic theory. Therefore, it would appear that under suitable conditions (defined below), an approximation of the quantity r_m with the use of demographic methods could potentially be used as an approximation of r_L in the logistic model.

Despite the conceptual overlap between logistic modeling and demographic analysis, the methodologies employed by each to estimate their parameters have remained separate. For example, in fisheries stock assessment, logistic models are typically fitted to abundance data to estimate r_L (hereafter referred to as r), and despite the availability of demographic data, no use has been made of demographic methods to facilitate this estimation. Demographic methods to approximate r_m are often overlooked in stock assessments or treated separately from the model fitting approach (Cortes 1998; Smith et al. 1998). In this paper, we illustrate the utility of demographic methods in helping to improve the accuracy of estimates of r for population modeling. Below, we review a few of the simpler demographic methods to approximate r_m and then provide some guidelines for the construction of a prior for r.

Demographic methods to approximate r_m

The simplest demographic methods for approximating r_o and r_m rely on estimates of "vital rates" such as fecundity at age and survival rate at age (from natural causes of death) (Krebs 1985; Begon et al. 1996). Estimates of these quantities are occasionally available for exploited populations. In such instances, demographic methods (Krebs 1985; Begon et al. 1996) can be applied to approximate r_m. It is important for practitioners to note when in the population's exploitation history the vital rates were estimated. To obtain approximations of r_m, the values for survival rate and fecundity should be obtained when density-dependent processes are least likely to be operating, for example, at abundances low relative to carrying capacity. Alternatively, if estimates are available when the population is moderately exploited, then it could be assumed that the abundance was near the MSYL. The realized rate of increase, r_r, of the population would then be less than the maximum possible value and would need to be increased by up to a factor of 2 if it were to be used as an approximation of r in the logistic model.

If the population is heavily exploited, it is unlikely that the population's age distribution will be stable, as required by some demographic methods. However, this should not bias demographic approximations of r_m provided that they do not obtain the survival rate at age directly from the observed age distribution, as in some cohort life table methods

(Begon et al. 1996). There are at least three simple demographic methods that could be used to approximate r_m.

Generation time method

The simplest method derives from the following approximation (Krebs 1985; Begon et al. 1996):

$$(3) \qquad R \approx 1\exp(r_m\overline{G})$$

where R is the expected lifetime contribution of female offspring from a single female organism and $\overline{G}$ is the approximated mean generation time. The $\overline{G}$ may also be interpreted as the weighted average age of reproductive females with weighting by the expected births at age after the survival rate at age of reproductive females has been accounted for (see eq. 8). The R can be obtained by

$$(4) \qquad R = \sum_{x=0}^{A} l_x m_x$$

where A is the maximum possible age of a female, l_x is the expected survivorship of females from age 0 to age x, and m_x is the expected number of age-0 female offspring per individual female or fecundity at age x. The r_m can thus be obtained by dividing the natural logarithm of the expected lifetime contribution of female offspring from a single female organism, R, by the mean generation time:

$$(5) \qquad r_m \cong \frac{\log\left(\sum_{x=0}^{A} l_x m_x\right)}{\overline{G}}$$

The components of this equation can be obtained as follows. The l_x is computed by

$$(6) \qquad l_x = \prod_{i=0}^{x-1} S_i$$

where S_i is the survival rate at age i and l_0 is 1. The m_x can be expressed as a function of the expected fecundity per individual female, sex ratio s, and the age at maturity:

$$(7) \qquad m_x = s g_x f_x$$

where g_x is the expected proportion of individuals mature at age x and f_x is the fecundity of those mature at age x. The arithmetic mean generation time, $\overline{G}_a$, which is typically applied, is approximated by

$$(8) \qquad \overline{G}_a = \frac{\sum_{x=0}^{A} x l_x m_x}{\sum_{x=0}^{A} l_x m_x}$$

with each age x weighted by the expected contribution of offspring at that age. Note that eq. 5 does not give the "true" value of r_m. The shape of the xl_xm_x versus x distribution influences how close the approximation of r_m from eq. 5 approaches the true value of r_m. If that distribution is not normal, this method does not work well (see Ebert (1999, pp. 17–18) for a discussion). Extensive simulations using the shark data below showed that this method and extensions to it (e.g., using the geometric and harmonic means instead of the arithmetic mean) were highly inaccurate and that the other two methods below should be used instead.

Leslie matrix method

A second approach to approximating r projects a Leslie population matrix over several time steps until the population age structure (proportion at age) has stabilized (Krebs 1985). The intrinsic rate of increase can then be computed from the ratio of abundance between one time step and the previous. The initial vector of female numbers at age can be initialized by

$$(9) \qquad N_{x,0} = 1000 l_x$$

The number of age-0 female individuals for the next time step is given by

$$(10) \qquad N_{0,t+1} = \sum_{x=0}^{A} m_x N_{x,t}$$

The number of female individuals of age x in the next step for ages 1 to A (assuming senescence at age A) is given by

$$(11) \qquad N_{x,t+1} = S_{x-1} N_{x-1,t}$$

Trial and error is required to identify when the age structure has stabilized. This usually does not take many time steps, and stabilization can be monitored by computing the average percent change in the proportion at age between each time step:

$$(12) \qquad \Delta_t = 100\frac{1}{A}\sum_{x=0}^{A} \frac{\frac{N_{x,t}}{P_t} - \frac{N_{x,t-1}}{P_{t-1}}}{\frac{N_{x,t-1}}{P_{t-1}}}$$

where

$$(13) \qquad P_t = \sum_{x=0}^{A} N_{x,t}$$

When this average percentage becomes very small, say <0.0001%, the age structure can be considered to be stable. Once age structure is stabilized, r_m can then be approximated from

$$(14) \qquad r_m = \log\left(\frac{P_t}{P_{t-1}}\right)$$

While this approach works well, the "classical" way to approximate r_m through Leslie or other matrices is using matrix algebra where $\lambda = e^{r_m}$ is the dominant eigenvalue of the transition matrix (Ebert 1999; Quinn and Deriso 1999). However, both the eigenvalue and matrix projection approaches are practically identical. We computed λ for the basecase for sandbar sharks (*Carcharhinus plumbeus*) below and, as expected, obtained results practically identical to those obtained using the matrix projection method. We present the simpler matrix projection approach rather than the eigenvalue one to make the methodology more accessible to a wider audience.

Euler–Lotka method

Thirdly, r_m can be approximated from the following equation (Lotka 1907):

$$(15a)\quad \sum_{x=0}^{A} e^{-r_m x} l_x m_x = 1$$

Note that this is a discrete approximation of an integral over ages 0 to ∞:

$$(15b)\quad \int_0^{\infty} e^{-r_m x} l_x m_x \mathrm{d}x = 1$$

The r_m cannot be solved analytically from this equation; it must be solved numerically using a computer. The initial guess for r can be that obtained by the first method above.

Steps for using demographic methods to construct a prior distribution for *r* in the logistic model

A prior distribution for r in the logistic model can be obtained from the following steps. (1) Formulate prior distributions for the rate of survival (S_x) or natural mortality at age (M_x) of female individuals and fecundity at age (m_x) of female offspring. (2) Randomly draw a vector of values from the prior distributions for S_x or M_x and m_x. Using one of the demographic methods described above, compute the value for r_m. (3) Repeat step 2 many times (e.g., ≥1000). (4) Eliminate all values for r_m that are less than a predefined minimum possible value (e.g., $r_m < 0.01$, see below). (5) Construct a frequency distribution of the resulting values for r_m. (6) Construct a parametric density function such as the beta or lognormal distribution based on the empirical distribution generated in steps 1 to 5. This parametric density function for r_m can be used as the prior for r in the logistic model. The formulation of a parametric density function is not absolutely necessary. One could instead work directly with the discrete frequency distribution obtained. While the above steps appear to be straightforward, we have several recommendations regarding them.

Use of the different demographic methods

Of the three demographic methods for approximating r_m, the generation time method (using the arithmetic mean generation time) is the simplest. It is also the least accurate but often provides values similar to those of the other methods. The inaccuracy results mainly because the arithmetic mean generation time (eq. 8) is a biased approximation of mean generation time; thus, other approximations of $\overline{G}$ are considered. In contrast, the Leslie projection and Euler–Lotka methods provide much more accurate approximations of r_m than the first method. We therefore recommend that these latter two methods for approximating r_m be applied and the results compared. To start, code up each of the methods in a spreadsheet and attempt to obtain comparable results with the same input parameters. We prefer the Leslie projection method because it provides a nearly exact numerical approach for approximation, providing that the simulated age structure has stabilized. In contrast, the Lotka method (eq. 15) is a discrete approximation of an integral over ages between 0 and ∞ (Lotka 1907). In particular, there may be inaccuracies in the approximation if survival rates are high and the maximum age is not very large.

Formulation of distributions for survival rates and fecundities

Because r in the logistic model is the rate of increase in abundance when density-dependent effects are nonexistent, the estimates of survival rate, fecundity, and proportion mature at age, S_x, f_x, and g_x (eqs. 6 and 7), used should only be those expected to occur at very low population size when density-dependent effects that can reduce survival rate and fecundity at larger population abundance are not operating. However, for many exploited populations, this should not be a serious problem, since a high percentage of them are overexploited (e.g., about 70% of assessed fish stocks (FAO 2000)).

In most instances, the most uncertain parameter is S_x, the survival rate at age, or, by transformation, M_x, the rate of natural mortality at age. A number of alternative distributions reflecting what is known about the survival rate at age should be formulated to evaluate the potential effects of uncertainty in this parameter on the distribution for r_m.

In some instances, distributions uniform over a small range, e.g., 0.8 to 1, for S_x have been formulated (NMFS 1996). We recommend that such distributions for S_x should be avoided. It is often the case that there is a most credible estimate of S_x and that credibility diminishes as the numerical distance from this value increases. Furthermore, narrow uniform distributions for S_x can produce distributions for r_m that have sharp cutoffs (see Results). Prior distributions that have sharp cutoff points can strongly bias estimation results, particularly if the true value is in the region of zero probability (Adkison and Peterman 1996).

Instead, we suggest alternative distributions for S_x such as the beta(v, w) (where $v > 0$, $w > 0$, mean $= v/(v + w)$, and variance $= vw/[(v + w)^2(v + w + 1)]$), and normal($\mu$, σ^2) truncated below 0 and above 1 (where μ is the median and σ is the standard deviation in the natural logarithm of the random variable) (Evans et al. 1993). A beta distribution for S_x is particularly convenient because this distribution falls between 0 and 1 and can take on a wide variety of shapes. A normal distribution truncated below 0 or a lognormal distribution for the rate of natural mortality at age, M_x, could also be appropriate whereby the median for M_x and the standard deviations in M_x or the natural logarithm of M_x, σ_x, are obtained empirically, if possible. If no empirical estimate for σ_x is available, then the value for this parameter could be set subjectively. For example, the minimum and maximum plausible values for M_x can be used to set the value for σ_x by associating these extremes with potential 95% confidence intervals for a lognormally distributed M_x. In Monte Carlo simulations, S_x can then be obtained by transforming the random variable M_x:

$$(16)\quad S_x = \exp(-M_x)$$

If the sensitivity of prior distributions for r to input distributions for M_x is to be evaluated, we recommend that at a minimum, variants to the baseline distribution for M_x be tried in which the median and σ_x are varied. If it is plausible that M_x or S_x varies with age, x, then priors for r should be constructed under this assumption.

Estimating r for population models in numbers versus biomass of individuals

The demographic methods that we describe are based on abundance in numbers as opposed to biomass. However, in many instances, the Schaefer equation models biomass, not numbers. How then can we obtain a prior for r using demographic methods? The answer is by applying the same methods. Appendix A proves that r is the same whether the modeling is in biomass or numbers, providing that weight at age remains constant from one generation to the next.

Estimating r for models of mature or exploited population abundance

The demographic methods to compute r_m are based on all age groupings (eqs. 3–15). In contrast, Schaefer models when applied in fisheries stock assessment apply to the age- and (or) size-classes vulnerable to fishing mortality. Appendix B proves that the value for r_m obtained by the demographic methods is independent of the age-classes included and modeled in the quantity P_t. Therefore, demographic methods for computing r_m are independent of the age or pattern of recruitment to the population modeled by the logistic model. However, the demographic methods applied will still need to include all of the possible age-classes.

Dealing with negative values of r_m

If there is considerable uncertainty over the survivorship and fecundity schedules, it is likely that some of the permutations of the combined fecundity and survivorship schedules that can be obtained from their respective distributions will result in negative values for r_m. However, it is highly unlikely for the intrinsic rate of increase in the logistic model to be equal to or less than zero, unless, for reasons other than exploitation, the population is destined for extinction. It could be argued that very small nonnegative values (e.g., $r < 0.01$) are also highly unlikely. This accounts for step 4 in the Monte Carlo procedure described above.

Illustrating the integration of demographic methods with Schaefer model assessments

Below, we illustrate the utility of integrating demographic methods in stock assessment using the Schaefer model with an application to data for large coastal sharks (LCSs) on the U.S. east coast. In 1996, the U.S. National Marine Fisheries Service (NMFS) held a Shark Evaluation Workshop (SEW) to evaluate the status of LCSs off of the east coast of the United States and recommend harvesting policies for this species grouping. The report of the workshop concluded that the LCS grouping was heavily depleted and suggested that at least a 50% cut in the catch of LCSs was required to promote population recovery. In 1997, the NMFS implemented a 50% cut in the total allowable catch (TAC) for commercial fishermen (Anonymous 1997). The recreational bag limit for Atlantic sharks was reduced to two fish per boat trip. Below, we describe how we carried out each of the five steps for Bayesian stock assessment (from McAllister and Kirkwood 1998*a*). The data for LCSs combined are used here instead of those for single species to make the results comparable with those obtained in the 1996 SEW.

Step 1 is to identify the alternative management procedures to evaluate. As in the 1996 SEW, we evaluated constant quota policies that were varying fractions of the 1995 total reported catch of LCSs (367 200 fish). The fractions varied from 100% to 90,..., 10, and 0%. These same policies were also evaluated in the 1996 SEW.

Step 2 is to identify the indices of policy performance. The indices used to evaluate policy performance include the following: (*a*) the posterior expected value for the ratio of stock abundance to carrying capacity ($E(N_{\mathrm{fin}}/K)$) in the years 2008, 2018, and 2028, (*b*) the probabilities that stock size in these three years will be larger than the stock size in 1998 ($P(N_{\mathrm{fin}} > N_{98})$), (*c*) the probability that the stock size in each of these years will be larger than $0.5K$, the MSY stock level ($P(N_{\mathrm{fin}} > 0.5K)$), and (*d*) the probability that stock size will be below $0.2K$ in these years ($P(N_{\mathrm{fin}} < 0.2K)$).

Steps 3 and 4 are to identify alternative hypotheses and evaluate the weight of evidence in their support. A key input to a decision analysis is the joint posterior probability distribution for the alternative hypotheses, e.g., alternative values for the estimated population model parameters. In this section, we describe the population dynamics model, the data, the likelihood function, the prior probability distributions, and the method to produce marginal posterior distributions for quantities of interest.

Population dynamics model and alternative hypotheses

The surplus production model that we applied, the same as in NMFS (1996), is in numbers rather than biomass and is detailed in Prager (1994). The surplus production function with fishing mortality included is given by

$$\text{(17)} \quad \frac{\mathrm{d}N_t}{\mathrm{d}t} = (r - F_t)N_t - \frac{r}{K}N_t^2$$

where t is the year, N is stock abundance, r is the intrinsic rate of increase, K is the carrying capacity, and F is the instantaneous fishing mortality rate. Dividing eq. 17 by N_t gives the realized rate of increase, r_r. The maximum harvest rate possible was assumed to be 0.99. Abundance is updated annually by

$$\text{(18)} \quad \begin{aligned} N_{t+1} &= \frac{\alpha_t N_t \exp(\alpha_t)}{\alpha_t + \beta N_t(\exp(\alpha_t) - 1)} \quad \text{when } \alpha_t \neq 0 \\ N_{t+1} &= \frac{N_t}{1 + \beta N_t} \quad \text{when } \alpha_t = 0 \end{aligned}$$

where $\alpha_t = r - F$ and $\beta = r/K$. The most cumbersome aspect of this version of the Schaefer model is that F_t can only be solved numerically (Prager 1994). Prager (1994, p. 376) provided two different equations in which F_t is on both sides of the equation for when $\alpha_t \neq 0$ and $\alpha_t = 0$. A starting guess was provided for F_t (the harvest rate C_t/N_t) and this was entered into the right-hand side of the equation. The resulting F_t was then computed. This latter F_t was input into the equation and this process was iterated until the difference between the inputted and outputted F_t was very small (<0.000001).

The alternative hypotheses considered in the stock assessment consisted of alternative values for the parameters r, K, and N_{75}/K. The initial uncertainty over these was represented by a joint prior pdf of r, K, and N_{75}/K, with each of the parameters independent.

Catch and catch per unit effort (CPUE) data

The production model used in NMFS (1996) was fitted to 13 different series of CPUE data. These data extend from 1975 to 1995. We fitted the same production model to these data and assumed that CPUE, $I_{j,t}$, was directly proportional to stock abundance.

$$(19) \quad I_{j,t} = q_j N_t$$

where q_j is the constant of proportionality or catchability coefficient. Note that this latter assumption may be inaccurate for several reasons. For example, there have probably been long-term changes in catchability and catchability may not be constant with stock size. However, for now, we ignore these possibilities.

The series of annual catches for LCSs extends from 1981 to 1996. All values but the last were taken from NMFS (1996). The value for 1996 was taken from the 1997 SEW. Note that some of the CPUE series extend well before the beginning of the catch series (from 1975) and that catches were taken even before 1975. It would have been desirable from the point of view of estimation for the catch and the CPUE series to begin at the same time and to begin as early as possible in the history of exploitation. Conventional estimation approaches only allow us to use data series beginning in 1981. However, the Bayesian approach can allow us to use the full CPUE series if we treat the catches between 1975 and 1980 as unobserved random variables. For simplicity, we have assumed that catches were constant over this period and equal to C_o and that C_o is an additional parameter to be estimated. We assumed that the total catches in 1997 and 1998 were given by the commercial quota (1285 mt divided by the average dressed weight of 18.2 kg·fish^{-1}) in 1997 plus 75% of the recreational harvest in 1996 and the values for longline and coastal discards for 1996 (NMFS 1997). The total came to 227 300 fish.

Likelihood function

In the likelihood function, we assumed that the data were lognormally distributed:

$$(20) \quad I_{j,t} \sim \text{lognormal}(q_j N_t, \sigma^2_{I_{j,t}})$$

where $\sigma^2_{I_{j,t}}$ is the annual variance in the lognormal density function and q_j is the constant of proportionality for series. In some of the CPUE series, annual estimates of the CV for sampling error were available ($\text{CV}_{j,t}$). The $CV^2_{j,t}$ were used as weights to determine the relative value for each annual $\sigma^2_{I_j}$:

$$\sigma^2_{I_{j,t}} = c_j \text{CV}^2_{j,t} \sigma^2_{I_j}$$

where c_j is the constant for series j required to make the weights sum to 1 (derived by solving for it algebraically) and $\sigma^2_{I_j}$ is the arithmetic mean value for the time series j variance. Here, CPUE data with relatively large sampling error variances were given less weight in the likelihood function (hence the term inverse variance weighting). The $\sigma^2_{I_j}$ and q_j for each CPUE series were treated as uncertain parameters but were integrated from the joint posterior distribution (Walters and Ludwig 1994), since they are of no direct interest to fishery management. The log likelihood function, λ, of the catch rate data was thus

$$(21) \quad \lambda = -\sum_{j=1}^{13} \sum_{t=1975}^{1995} \frac{0.5}{c_j \text{CV}^2_{j,t} \sigma^2_{I_j}} \left[\log\left(\frac{I_{j,t}}{q_j N_t} \right) \right]^2 - 0.5 \log(c_j \text{CV}^2_{j,t} \sigma^2_{I_j})$$

Prior probability distributions

Prior probability distributions were required for r, K, N_{75}/K, C_o, q_j, and σ_{I_j}. Prior probability distributions were informative for r, N_{75}/K, and C_o only. The noninformative prior distributions assumed for q_j and σ_{I_j} were uniform over the natural logarithm of these quantities, and this made their integration from the joint distribution extremely simple (see Walters and Ludwig (1994) for the numerical shortcut).

The informative prior for r for LCSs was developed using demographic information for the two species most common in the LCS fishery, sandbar shark and blacktip shark (*Carcharhinus limbatus*). The input distributions formulated were based on those given in NMFS (1996). In NMFS (1996), a Monte Carlo method similar to that described above was applied to compute probability distributions for r_m based on input distributions for survivorship at age and pupping rate at age for sandbar sharks. For example, in one instance, the maximum age, A, was considered to be 100 years, the age at maturity was uniformly distributed between, and including, 13 and 17 years, the fecundity at age was normal with a mean of 2.1 female pups per year and a CV of 0.3, and the annual survival rate was uniformly distributed between 0.8 and 1.0 and assumed to be constant with age. This latter assumption is unrealistic and the survival rate from natural causes of death is typically much less for the youngest age-classes. Assuming constant survival rate instead could give rise to positively biased approximations of r_m. We applied this same assumption in this paper to replicate as closely as possible the results in the 1996 stock assessment (NMFS 1996). Moreover, for sharks that produce well-developed, free-swimming young, the assumption of constant survival rate at age is unlikely to result in large biases. On the other hand, the values used for parameters such as S_x were obtained not at the very lowest abundances and this should compensate to some extent for the positive bias caused by assuming constant S_x. For reasons described above, narrow uniform distributions for parameters such as survival rates are better replaced by beta distributions for S_x or lognormal distributions for the rate of natural mortality.

With these and other input distributions (NMFS 1996), the resulting output distributions for r include values mostly above zero, although in all cases, and others that we tried, the lower tail of the output distributions always included negative values. The Monte Carlo procedure outlined above suggests that the resulting distribution for the intrinsic rate of increase be truncated at or just above zero. The median values for r_m in the distributions from NMFS (1996) ranged between 0.07 and 0.11, and the standard deviations (SDs) ranged between 0.03 and 0.05 (NMFS 1996). In contrast, in the 1996 SEW, the frequentist estimation method that was used to fit the Prager model to CPUE data to estimate r and K ignored these demographic results and obtained a maximum likelihood estimate of 0.26 (highly imprecise largely due to the one-way trip in the CPUE data). To obtain potentially more reliable estimates, the Bayesian approach allows us to formulate a prior distribution based on the demographic results that can be combined with the likelihood function of the data.

We computed prior distributions for r for sandbar sharks using a variety of alternative input distributions for S_x assumed to be constant with age x (Fig. 1). These included (*i*) S_x uniform on 0.8, 1, (*ii*) S_x in a symmetric triangular distribution with minimum at 0.8 and maximum at 1.0, (*iii*) S_x in a distribution with minimum at 0.8, maximum at 1.0, and a parabolic tail at each end of the distribution so that the most likely region was between 0.85 and 0.95, (*iv*) $S_x \sim \text{beta}(54, 6)$, (*v*) $S_x \sim \text{beta}(18, 2)$, (*vi*) $M_x \sim \text{lognormal}(-\log(0.9), 0.4^2)$, and (*vii*) $M_x \sim \text{lognormal}(-\log(0.9), 0.7^2)$. In the latter two distributions, the term $-\log(0.9)$ gives the same median value of 0.9 for S_x as in the former four distributions. Moreover, the latter two lognormal distributions also give distributions for S_x analogous to the two beta distributions (Fig. 1) (see Evans et al. (1993) for methods to draw random variables from these distributions). We also provide results for r_m for blacktip sharks when the age at maturity is triangular between and not including 5 and 9 years of age with a mode at 7, A of 20, fecundity at age ~ normal$(2.5, 0.75^2)$, and $M_x \sim \text{lognormal}(-\log(0.9), 0.7^2)$ for ages 1+ and the same distribution with a median survival rate of 0.5 for age 0 (E. Cortes, NMFS, 3500 Delwood Beach Road, Panama City, FL 32408, U.S.A., personal communication).

As mentioned above, a convenient density function for r is the lognormal, since values less than zero are impossible and it is skewed to the right, indicating that most of the density centers around lower values. The SD in the logarithm of r (σ_r) for the lognormal density function is given by

$$\text{(22)} \quad \sigma_r = \sqrt{\ln\left(1 + \left(\frac{\text{SD}(r)}{\text{mean}(r)}\right)^2\right)}$$

Using as baseline values a mean of 0.07 and an SD of 0.05, the result is $\sigma_r = 0.64$. However, due to the variation in the median value for r among the sensitivity tests in the demographic analysis, we rounded σ_r to 0.7.

The prior for K was uniform over the natural logarithm of K for values of K from 1000 to a trillion individuals. This implies that within this range the prior probability for K is proportional to $1/K$. This prior gives lower probability to higher values for K and makes values of K less than or equal to zero impossible. The value for N_{75} was set equal to ηK where η has a mean of 1 and an SD of 0.2 and is lognormally distributed. This prior reduces the possibility that N_{75} will be much higher than K due to imprecise or biased data. The prior for the mean catch in years 1975–1980 (C_0) was lognormally distributed with a mean equal to the mean of the observed catches (477 000). The SD in the natural logarithm of observed catches was 0.30 (eq. 22); however, we set the prior SD in the natural logarithm of C_0 to 0.5 to account for the fact that C_0 represented catches outside of the observed catch time series.

Integrating the joint posterior distribution to produce marginal posterior distributions

The parameters q_j and σ_{I_j} were integrated from the joint posterior of θ using the method of Walters and Ludwig (1994). The joint posterior distribution for the remaining parameters, r, K, C_0, and η (a vector called θ''), was integrated to produce marginal posterior distributions and mean values for these and other model quantities using the sampling/importance resampling algorithm (Rubin 1988; McAllister et al. 1994; McAllister and Ianelli 1997). For situations with up to a few dozen parameters, this can be a simple and highly efficient Monte Carlo method for Bayesian integration. Samples of parameter values, θ, are randomly drawn from an importance function. This is a pdf of θ constructed to be as similar as possible to the actual posterior density function of interest but with tails no sharper than those of the actual posterior pdf. A weighting factor is computed for each sample of θ that is proportional to the posterior density. It can be easily shown that the resulting distribution of θ converges on the posterior density function by the strong law of large numbers (Berger 1985). It took up to 300 000 draws from the importance function (up to about 10 min on a 300-MHz Pentium desktop) to achieve very good approximations of the estimated marginal posterior distributions. The stopping rule applied was to stop the run when the number of draws from the importance function exceeded 20 000 and the maximum importance weight in all cases had dropped to below 1% of the total summed importance weights (if draws were taken from the posterior, this percentage would be $100\% \cdot n^{-1}$ where n is the number of draws from the importance function). For other diagnostics, see McAllister and Ianelli (1997).

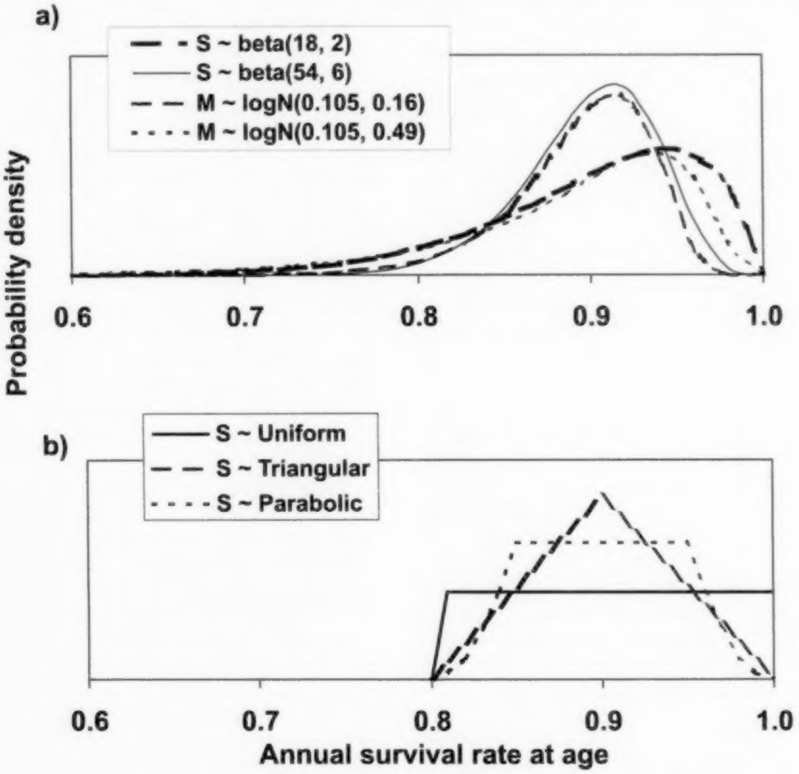
We tested the sensitivity of the marginal posterior distributions for the key model parameters, for example, r, K, N_{95}, and N_{98}/K, and the maximum sustainable yield (MSY = $rK/4$) to alternative prior distributions for r. Step 5 is to evaluate the distribution and expected value of each performance measure. In order to calculate the posterior expected value for the various indices of policy performance, the following steps were applied. (1) Randomly draw 5000 vectors of parameters θ'' (θ'' = r, K, C_0, and $\eta = N_{75}/K$) with replacement from the discrete approximation to the posterior distribution of θ'' with the probability of drawing each vector θ''_j being proportional to the posterior probability $P(\theta''_j|\text{CUPE})$. This is the resampling step in the sampling/importance resampling algorithm (Rubin 1988; McAllister et al. 1994; McAllister and Ianelli 1997) to obtain a set of draws of θ'' from their joint posterior distribution. See McAllister and Kirkwood (1998*a*) for an algorithm for randomly drawing values from such a distribution. (2) Using each drawn vector θ''_j, project the surplus production model (eq. 18) from the year 1975 to 1998. Then project the model from 1998 to 2028 and apply one of the constant quota policy options in each year. (3) In each projection j and year y, calculate the values for MSY$_j$ and $B_{j,y}/K_j$ and check to see if it drops below $0.2K_j$ in the years 2008, 2018, and 2028 or increases above $0.5K_j$ or $B_{j,98}/K_j$ in each of these years. If the condition has been met at least once in the projection, then add 1 to the sum for that policy index. (4) To obtain the expected value for each other policy index, divide the summation of values for each index by 5000.

Results

Prior distributions for r

We first compare prior distributions obtained using two

Fig. 1. Input prior distributions for the annual rate of survival (S) based on (a) beta distributions for S and lognormal distributions for the rate of natural mortality (M) and (b) uniform, triangular, and parabolic distributions for S.



different demographic methods to compute approximations of r_m for sandbar sharks. Without the elimination of low values for r_m from the Monte Carlo procedure, about 12% of the simulated values fell below zero for each of the methods (Fig. 2a). Because such values are biologically impossible, it was necessary to introduce a lower cutoff point for plausible values of r_m. All subsequent results were obtained using a lower cutoff point of $r_m = 0.01$.

The correlation between values of r_m between the Lotka and Leslie matrix methods was >99.99% (Fig. 2). However, the approximations provided by the Lotka method were consistently about 5% larger than estimates from the Leslie matrix method (Table 1). The SDs in estimates from the Lotka method were also consistently about 5% larger. The reasons for these discrepancies are not clear but could result because the Lotka method applied is a discrete approximation of an integral over the ages from 0 to ∞, while the Leslie method is more of an exact numerical approximation that directly simulates the rate of population increase (Lotka 1907).

The shape of the distribution for r_m was relatively insensitive to the precise functional form of the input distribution assumed for S_x (Fig. 3), provided that the input distribution for S_x had a similar central tendency and spread. The uniform distribution for S_x over 0.8, 1.0 produced the broadest distribution for r_m with the sharpest cutoff points (median r_m (r_{m50}) = 0.09, SD(r_m) = 0.047; from the Leslie method) (Fig. 3a; Table 1). When the parabolic and triangular distributions for S_x were applied, which had less spread than the uniform distribution for S_x, the distribution for r_m had more gradual tails (for both, $r_{m50} = 0.07$ and SD(r_m) = 0.038) (Table 1). The priors based on the beta distributions for S_x tended to be skewed slightly to the right, and the spread of the distribution for r_m depended strongly on the parameterization of the beta distribution (Fig. 3b). For S_x ~ beta(18, 2), $r_{m50} = 0.09$ and SD(r_m) = 0.043 and for S_x ~ beta(54, 6), $r_{m50} = 0.07$ and SD(r_m) = 0.033 (Table 1). The two lognormal distributions for M_x, were parameterized to give distributions for S_x similar in shape to those for the two beta distributions and therefore gave distributions for r_m similar to those for the beta distributions for S_x (Figs. 3b and 3c; Table 1). A lognormal prior distribution for r_m that incorporates a median of 0.07 and lognormal SD of 0.5 tends to weight the smaller values for r_m more heavily and has a more gradually tapering upper tail than all of the distributions derived from the Monte Carlo exercise (Fig. 3).

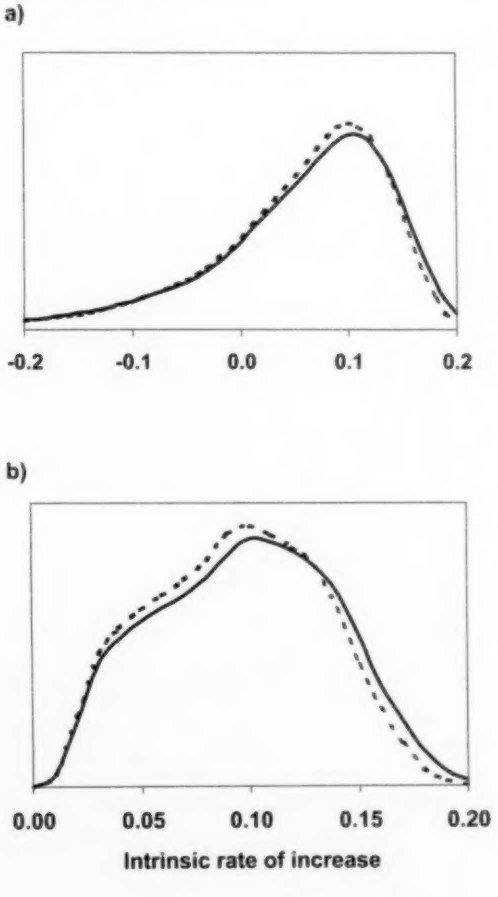
Similar biases in the distributions produced by the different methods to calculate r_m were observed when the input distributions for blacktip sharks were applied (Fig. 3d; Table 1). The Lotka method gave slightly higher values for r_m than the Leslie method (Table 1). The prior distribution produced for blacktip sharks had a higher central tendency ($r_{m50} = 0.125$, SD(r_m) = 0.059) than the one produced for sandbar sharks, mainly because the age at maturity was much lower for blacktips (Fig. 3e; Table 1). A prior for r in the logistic model for LCSs that gives less productive species more weight but has a larger SD to account for the vari-

Table 1. Description of priors for r resulting from different input distributions for survival rate at age.

Shark species	Distribution	Method	r_{m50}	$\bar{r}_m$	SD(r_m)	CV(r_m)	σ_r
Sandbar	$M \sim$ lognormal(0.105, 0.7^2)	Leslie	0.085	0.083	0.038	0.46	0.43
		Lotka	0.089	0.088	0.040	0.46	0.44
	$M \sim$ lognormal(0.105, 0.4^2)	Leslie	0.071	0.070	0.032	0.45	0.43
	$S \sim$ beta(18, 2)	Leslie	0.089	0.090	0.043	0.48	0.46
	$S \sim$ beta(54, 6)	Leslie	0.073	0.074	0.033	0.45	0.43
	$S \sim U$(0.8, 1)	Leslie	0.090	0.089	0.047	0.53	0.49
	$S \sim$ triangular(0.8, 0.9, 1)	Leslie	0.070	0.074	0.038	0.51	0.48
	$S \sim$ parabolic(0.8, 0.85, 0.95, 1)	Leslie	0.074	0.075	0.038	0.51	0.48
Blacktip	$M_0 \sim$ lognormal(0.693, 0.7^2) $M_{1+} \sim$ lognormal(0.105, 0.7^2)	Leslie/Lotka	0.125	0.137	0.059	0.32	0.32

Note: r_{m50}, median r_m; $\bar{r}_m$, mean r_m; SD(r_m), standard deviation for r_m; CV(r_m), coefficient of variation in r_m; σ_r lognormal standard deviation for r_m.

Fig. 2. Prior pdfs for r for sandbar sharks produced using the Leslie matrix (dashed line) and Lotka (solid line) methods. The age at maturity was assumed to be uniformly distributed between 14 and 17 years, the maximum age was 100, fecundity at age was normal(2.1, 0.63^2), and M_x was lognormal(0.105, 0.7^2) (baseline and used in subsequent figures). (*a*) Distributions without any truncation below the value of 0.01; (*b*) distributions with truncation below the value of 0.01.



ability in r among species in this grouping is given by a lognormal distribution with a median of 0.07 and a lognormal SD of 0.7 (Fig. 3*e*).

Posterior distributions for logistic model parameters

A joint posterior pdf for r and K in the logistic model from a noninformative (uniform) prior for r is shown in Fig. 4*a*. This demonstrates that there is relatively little information about r and K in the data, which is consistent with a "one-way trip" (Fig. 4*c*). A long narrow ridge of higher probability runs from high values for r and low values for K to lower values for r and higher values for K. Along this ridge, many different combinations of values for r and K result in model predictions that correspond almost equally well to the CPUE data. However, because the ridge of higher probability is relatively narrow and runs diagonally across the joint region for r and K, it can be seen that if the value for r were known, the estimate of K would be considerably more precise and vice versa. Using noninformative priors and assumptions similar to those in the 1996 assessment, we were, however, unable to obtain estimates of r anywhere close to that obtained in NMFS (1996) (0.26). For example, our posterior mean with a noninformative prior on r was 0.7 with an SD of about 0.2 (Table 2). Although the posterior CVs for most quantities were similar when an informative prior for r was used (Table 2), the posterior SDs were larger for most quantities when the uniform prior was applied. Curiously, the posterior CV for MSY was only 5% with the uniform on r prior compared with 44% in the baseline case. This resulted because the posterior correlation between r and K was –1 with a uniform prior on r and –0.59 with an informative prior. The use of an informative prior for r rather than a noninformative prior thus produces marginal posteriors that are not necessarily more informative about model quantities (Table 2; Figs. 4*b* and 5). The most noteworthy difference is in the central tendencies of the marginal posteriors.

Under the baseline case with the informative prior for r, the posterior CV for r (62%) is only slightly smaller than the prior CV (80%), also indicating that the data are not informative for the parameter r (Fig. 5*b*). The posterior expected value for catch (309 000 fish, CV = 44%) for the years 1975–1980 ($E(C_0)$) was less than the prior expected value (477 000 fish), indicating that the data suggest a smaller value for this quantity (Fig. 5*c*). Despite the broad posteriors

Fig. 3. Histograms of r_m for sandbar sharks produced by using distribution for (*a*) $S_x \sim$ uniform(0.8, 1.0), $S_x \sim$ triangular(0.8, 0.9, 1.0), and $S_x \sim$ parabolic in the tails with the minimum and maximum values at 0.8 and 1.0, respectively, and with S_x uniform between 0.85 and 0.95, (*b*) $S_x \sim$ beta(18, 2) and $S_x \sim$ beta(54, 6), and (*c*) M_x that was lognormal(0.105, 0.4^2) and M_x that was lognormal(0.105, 0.7^2); a parametric prior distribution for r that is lognormal(0.07, 0.5^2) is also given in Figs. 3*a*–3*c*; (*d*) histograms for r_m for blacktip (BT) sharks from the Leslie projection and Lotka methods; (*e*) prior for r for blacktip sharks from the Leslie method, priors for r for blacktip and sandbar (SB) sharks, both produced by fitting a lognormal distribution to the Monte Carlo results, and a prior for r for LCSs that gives less productive species more weight but has a larger SD to account for the variability in r among species in this grouping.

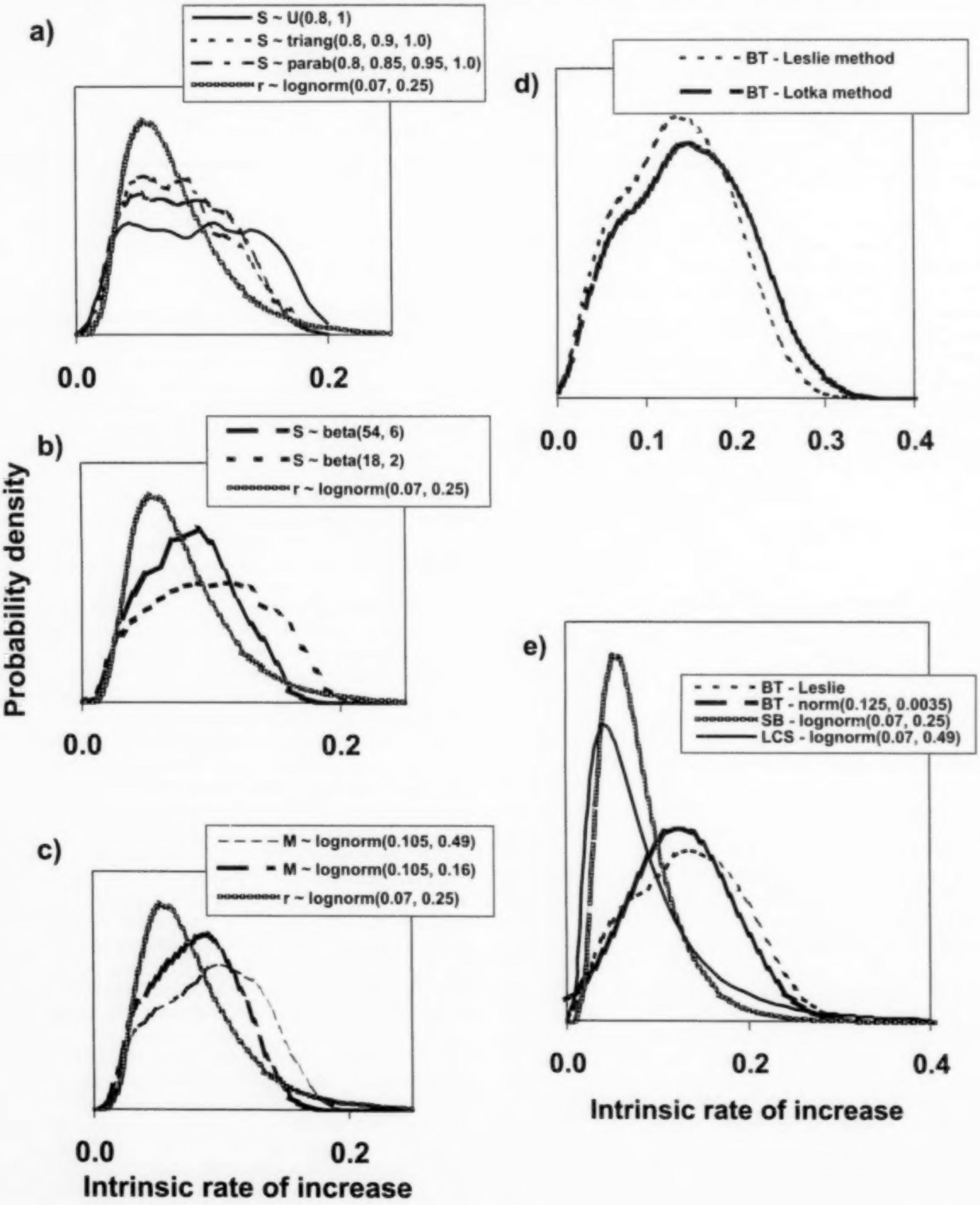
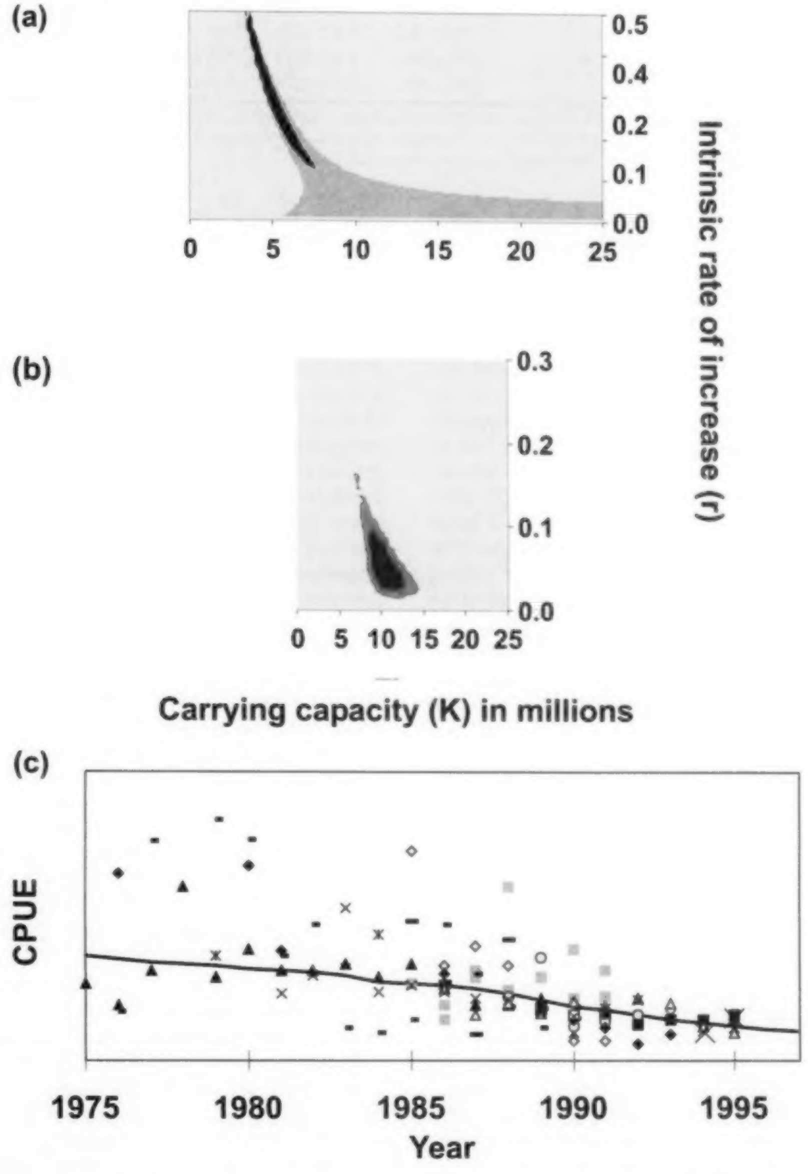


Fig. 4. (*a*) Joint log likelihood profile for r and K in the logistic model; (*b*) joint posterior distribution for parameters r and K using the baseline priors: prior median for $r = 0.07$, $p(K) \propto 1/K$, prior mean$(C_0) = 477\,000$ fish, prior SD[log C_0] = 0.52, prior mean(N_{75}/K) = 1, and prior SD[log(N_{75}/K)] = 0.2; (*c*) plots of the 13 CPUE series (rescaled to abundance in the baseline assessment) (symbols) and the modal posterior trend in abundance (line).



in model parameters, the data suggest a strong decreasing trend in abundance since the mid-1970s (Fig. 6). The marginal posterior distribution for abundance in 1998 is markedly lower than that for abundance in 1975 with very little overlap between these distributions (Fig. 5*d*).

Sensitivity of results to alternative priors

In Bayesian stock assessment, it is common to evaluate the sensitivity of results to alternative prior distributions for model input parameters. When the prior median value for r was increased to 0.11 and 0.20, $E(r)$ also increased, $E(K)$ decreased, and $E(N_{98}/K)$ and $E(\mathrm{MSY})$ increased (Table 2; Fig. 7). The posterior CV for r remained the same. $E(\mathrm{MSY})$ was particularly sensitive to the prior for r. When the prior median for r was increased from 0.07 to 0.11 and 0.20, $E(\mathrm{MSY})$ increased from 157 000 (CV = 0.44) to 205 000 (CV = 0.39) and 284 000 fish (CV = 0.30).

Decision analysis results

Using a uniform prior for r provided very optimistic pre-

Table 2. Posterior means and % CVs (in parentheses) for various management quantities for U.S. LCSs.

	Expected value					
Priors (all same as baseline except for these changes)	K	r	C_o	MSY	N_{98}	N_{98}/K
Uniform prior for r	2 868 (24)	0.69 (28)	224 (33)	462 (5)	1274 (27)	0.45 (11)
Baseline (median r = 0.07, etc.)	10 226 (21)	0.07 (62)	309 (44)	157 (44)	2964 (32)	0.29 (29)
Median r = 0.11	9 142 (23)	0.10 (62)	295 (44)	205 (39)	2804 (34)	0.31 (29)
Median r = 0.20 (mean = 0.26)	7 339 (30)	0.19 (62)	268 (46)	284 (30)	2511 (36)	0.35 (30)

Note: C_o is the mean annual catch before 1981. All runs used the CPUE data from NMFS (1996, 1997), and the 13 CPUE series were not combined. Baseline refers to the use of a prior that is lognormal(0.07, 0.7^2) for r, uniform on log(K), lognormal(477, 0.52^2) for C_o, and lognormal(exp($-0.2^2/2$), 0.2^2) for the ratio of N_{75} to K. Abundance values (i.e., K, MSY, and N_{98}) are in thousands.

dictions of resilience to exploitation (Table 3). With a low in abundance of 37%K in 1993 and a TAC continued at the 1995 level, recovery to the MSYL was predicted by 1999. In contrast, when the baseline posterior distribution, which incorporates a prior for r based on demographic analysis, is applied in the decision analysis, TACs similar to the 1998 TAC (about 60% of the 1995 TAC) for LCSs are not sustainable. When f was maintained at the 1998 level of approximately 60% for the next 10 years, $E(N_{\text{fin}}/K)$ dropped to 0.19. Only a policy in which the TAC was set to $f \leq 30\%$ of the harvest in 1995 had more than a 50% chance of increasing stock size above the 1998 level (Fig. 8; Table 3). Only values for f that were 10% and smaller resulted in a larger than 50% chance of increasing stock size to larger than 0.5K after 30 years (Fig. 8). A complete elimination of fishing mortality could only result in a reasonable chance of stock recovery after 20 years (Table 3; Fig. 8).

Increasing the prior median value for r markedly increased the chance of recovery for the harvesting options considered. For example, with the prior median for r set to 0.11, values for $f \leq 40\%$ had more than a 50% chance of increasing stock size over the 1998 level after 30 years (Table 3). With the prior median for r set to 0.20, values for $f \leq 60\%$ had more than a 50% chance of increasing stock size above the 1998 level after 30 years (Table 3).

Discussion

Much of the published work on Bayesian stock assessment applies age-structured models that incorporate detailed age-structured data and information (McAllister and Ianelli 1997; Punt and Hilborn 1997; Patterson 1999). However, more highly aggregated models, such as a surplus production model, can often be suitable when less detailed data are available. But even for these latter models, priors are still required and relatively little attention has been given to this. Hoenig et al. (1994) provided an empirical Bayes method for constructing a prior distribution for parameters of an equilibrium surplus production model using data from several subpopulations. However, for a variety of reasons, equilibrium models are often inappropriate (Hilborn and Walters 1992).

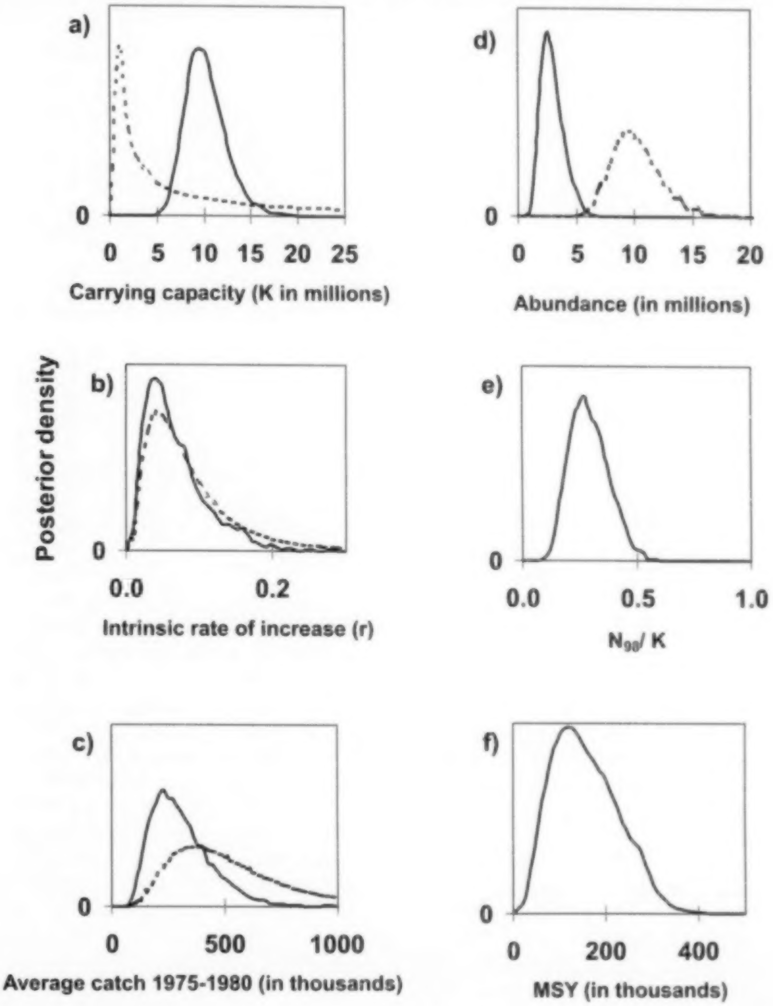
Kinas (1996) applied adaptive importance sampling, a method for Bayesian integration, to estimate the parameters in a nonequilibrium Schaefer model for orange roughy (*Hoplostethus atlanticus*). Because the application was primarily to illustrate methods of Bayesian integration, little attention was given to the construction of priors, e.g., for the intrinsic rate of increase. Unlike the current study, the optimal harvest decision was the same for diffuse and informative priors. McAllister and Kirkwood (1998*a*) also exemplified a framework for Bayesian stock assessment using a nonequilibrium Schaefer model. Using catch and CPUE data for cape hake (*Merluccius capensis*), they demonstrated that CPUE data could only be informative about r if the CPUE data showed a decreasing trend followed by an increasing trend in response to a reduction in fishing effort. McAllister and Kirkwood (1998*b*) also applied a nonequilibrium Schaefer model to evaluate alternative management procedures for newly developing fisheries. They demonstrated that risks of overdepletion could increase if priors for uncertain model parameters were specified too precisely (e.g., prior CV < 0.5). Meyer and Millar (1999) exemplified useful and readily accessible software for Bayesian data analysis, BUGS, with a Schaefer surplus production model. A state–space modeling framework is advocated that provides a conceptually and operationally straightforward way to deal with model process error and observation error within a single unified analytical framework. A Markov chain Monte Carlo method is used for numerical integration in contrast with the sampling/importance resampling approach in this paper. Each alternative integration approach has its conveniences (McAllister and Ianelli 1997), and at least for fairly simple stock assessment problems such as the one in this paper, the choice is a matter of personal preference. While the latter four works above give scarce attention to the construction of prior distributions for Schaefer model parameters, they could potentially use the methods for this presented in this paper.

Linking demographic methods with parameter estimation for Schaefer models

In this paper, we apply Bayesian methods to link demographic analysis with the fitting of surplus production models to time series data to improve the empirical basis for parameter estimation. The theoretical basis for this link was presented first. Here, we summarized three simple approaches to approximating r_m from the same age-structured information, the mean generation time, Leslie matrix, and Lotka methods. We showed that r_m in demographic analysis is independent of whether the population is modeled in biomass or numbers and that r_m is independent of the age-classes that make up the modeled population. We also showed that owing to conceptual similarities, the r_m calculated using demographic methods can be exchangeable with r in surplus production models in numbers and biomass and is independent of the part of the population modeled.

We presented a Monte Carlo approach to constructing a

Fig. 5. Baseline marginal posteriors for (*a*) K, (*b*) r, and (*c*) C_o. The prior is the dashed line and the posterior is the solid line. Baseline marginal posteriors for (*d*) abundance in 1975 (dashed line) and 1998 (solid line), (*e*) N_{98}/K, and (*f*) MSY.



prior distribution for r in the logistic model from demographic analysis and some alternative methods to account for uncertainty in inputs to demographic analysis such as survival rate at age. The application to LCSs illustrates how age-structured demographic information can be integrated into the estimation of parameters in the Schaefer model to increase accuracy in parameter estimates and provide biologically sensible estimates for the evaluation of stock status and alternative fishery management plans for stock rebuilding. The methods rely on estimates of natural mortality, fecundity, and the ages of maturity and senescence. Biological research to obtain these estimates is essential before demographic methods can provide useful results.

The results for both sandbar and blacktip sharks supported the observation in textbooks that the Lotka and Leslie matrix methods for approximating r_m give almost the same results (Krebs 1985; Begon et al. 1996). However, for both blacktip and sandbar sharks, the Lotka method gave results consistently 5% higher and with larger variance than the Leslie method. As mentioned above, we believe that bias is more likely in the Lotka method because the Lotka method is a discrete approximation of an integral over an infinite range of ages, and the model applied assumes senescence yet allows the possibility of very high survival rates (Lotka 1907). We therefore favor the use of the Leslie method, which does not require such an approximation. Although this is computationally more intense, it takes only a minute or two on a modern desktop computer to generate a prior distribution using the Leslie method. Other methods such as eigenvalue methods (Cortes 1998; Ebert 1999; Quinn and Deriso 1999) could also be used, but it would be advisable to cross-check the results by applying other demographic methods to the same data.

Additional work not reported here demonstrated that the mean generation time method for computing r_m could sometimes result in very large negative biases in approximations of r_m. The replacement of the arithmetic mean with the harmonic mean helped to reduce this bias, but not entirely. In

Table 3. Consequences of alternative harvesting policies to promote resource recovery.

$f = \%C_{95}$	N_{fin}/K	$P(N_{\text{fin}} < 0.2K)$	$P(N_{\text{fin}} > 0.5K)$	$P(N_{\text{fin}} > N_{98})$
Baseline[a]				
0	0.67	0.00	0.76	1.00
10	0.58	0.04	0.60	0.95
20	0.47	0.17	0.45	0.74
30	0.36	0.36	0.31	0.52
40	0.25	0.55	0.21	0.34
50	0.16	0.71	0.13	0.20
60	0.09 (0.86)	0.83 (0.01)	0.07 (0.99)	0.10 (0.99)
70	0.05	0.91	0.04	0.05
80	0.03	0.95	0.01	0.02
90	0.02	0.98	0.01	0.01
100	0.01 (0.72)	0.99 (0.01)	0.00 (0.99)	0.00 (0.99)
$r_{50} = 0.11$				
0	0.78	0.00	0.89	1.00
10	0.71	0.01	0.80	0.99
20	0.61	0.08	0.67	0.89
30	0.51	0.20	0.53	0.72
40	0.39	0.36	0.40	0.55
50	0.28	0.52	0.28	0.38
60	0.20	0.66	0.19	0.25
70	0.13	0.78	0.12	0.16
80	0.08	0.87	0.08	0.09
90	0.05	0.92	0.05	0.05
100	0.03	0.96	0.02	0.02
$r_{50} = 0.20$				
0	0.92	0.00	0.99	1.00
10	0.87	0.00	0.97	1.00
20	0.81	0.00	0.91	1.00
30	0.74	0.04	0.84	0.93
40	0.65	0.12	0.74	0.84
50	0.55	0.22	0.63	0.72
60	0.45	0.35	0.53	0.59
70	0.36	0.45	0.43	0.48
80	0.28	0.57	0.34	0.37
90	0.20	0.68	0.24	0.27
100	0.14	0.78	0.15	0.16

Note: N_{fin}/K, stock abundance in the final year (2028) of management as a percentage of K; $P(N_{\text{fin}} < 0.2K)$, probability that stock abundance falls below $0.2K$ in the final year of the management horizon; $P(N_{\text{fin}} > 0.5K)$, probability that stock abundance will be above $0.5K$ in the final year of the management horizon; $P(N_{\text{fin}} > N_{98})$, probability that stock abundance in the final year of the management horizon will be above that in 1998; $f = \%C_{95}$, TAC policy option evaluated. The percent values in the last column reflect the TAC evaluated as a percentage of the total reported catch in 1995 (367 200 fish).

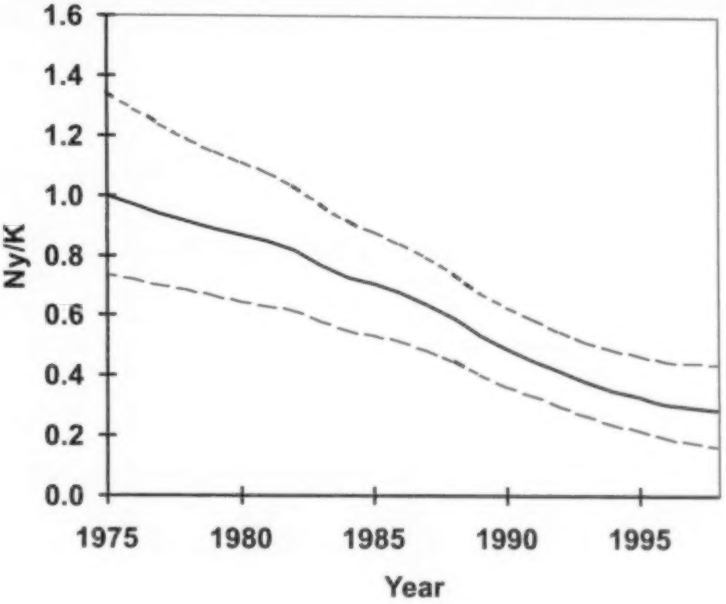
[a] The values in parentheses were obtained when a uniform prior for r was used in a separate decision analysis and are included in this table to facilitate comparisons.

contrast, textbooks typically show examples in which the results provided by the Lotka and mean generation time methods give very similar results (Krebs 1985; Begon et al. 1996). We recommend that practitioners avoid use of the mean generation time method even if the harmonic instead of arithmetic mean generation time, with its smaller bias, is applied.

The example also demonstrates that the probabilistic framework for logistic model parameter estimation can be used to indicate the effect of different assumptions about model input parameters on the estimates of, and uncertainties in, management quantities of interest such as MSY and the current level of stock depletion.

The approach that we apply to construct a prior distribution for r is analogous to other methods that have applied Monte Carlo methods to construct prior distributions that combine empirical information and expert judgment (McAllister et al. 1994; McAllister and Ianelli 1997). However, the simple Monte Carlo approach developed in this paper is more reliant on empirical information, for example, about life history parameters, than these other Monte Carlo approaches, which, for example, rely on expert judgment for the specification of input distributions for key sources of uncertainty in acoustic and trawl survey abundance estimates. An alternative approach to these is meta-analysis with hierarchical probability models (Gelman et al. 1995; Liermann and Hilborn 1997). This approach instead assumes that the

Fig. 6. Abundance relative to *K* between 1975 and 1998 (solid line) and 95% probability intervals (dashed lines).



parameter(s) of interest varies across similar populations and incorporates data from other similar populations to estimate the form of this distribution. The approach in Myers et al. (1997, 1999) has been applied to construct a prior distribution for r_m for albacore (*Thunnus alalunga*) using stock–recruit data from 12 other tuna populations (Millar and Meyer 2000). However, hierarchical meta-analysis is the most data-intensive method for developing priors and, as with sharks, the data required are often not available. Where both stock–recruit data from other populations and demographic data for the population of interest are available, hierarchical meta-analysis and the demographic method in this paper could both be applied to construct alternative prior distributions for r, and the sensitivity of decision analysis results to these alternative priors could be communicated to decision makers. Another option is to apply meta-analysis to abundance data from taxonomically similar very heavily fished populations where exploitation has been stopped and to approximate a pdf for r from the rebounds in observed abundance (Best 1993).

Comparisons between Bayesian and non-Bayesian results

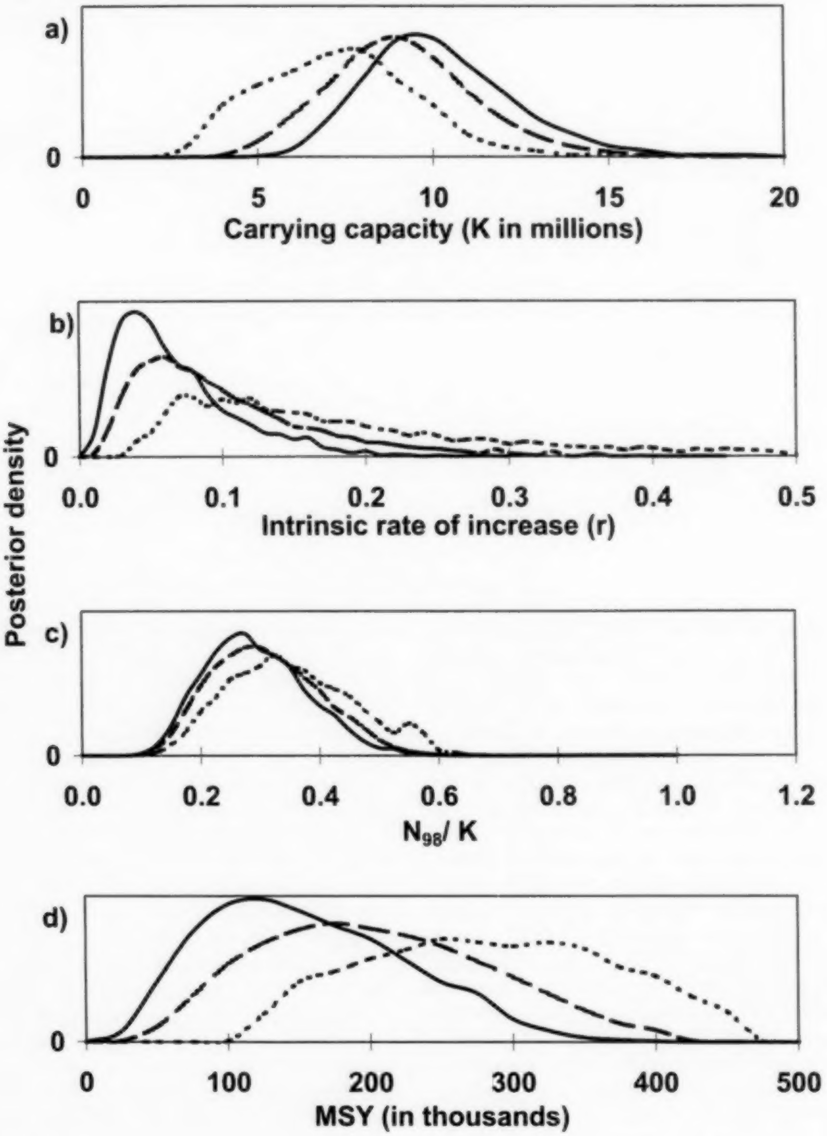
The Bayesian assessment results on the status of LCSs are far less optimistic than those in a recent non-Bayesian assessment (NMFS 1996) that used the same surplus production model and catch and CPUE data (we note that for the 1998 assessment, the NMFS adopted the approach presented in this paper (NMFS 1998)). The Bayesian estimate of the historic decline in abundance is not dissimilar from that in the 1996 SEW. For example, the 1996 SEW indicated that LCS abundance had declined about 50–75% between the 1970s and 1996. The baseline analysis indicates that abundance appears to have declined about 55–80% over the same time period. This similarity is not surprising, since the same model was fitted to the same CPUE data.

A major difference is in the estimate of r in the Prager (1994) production model. The non-Bayesian analysis in NMFS (1996) provided a production model estimate of r of 0.26. This estimate ignored "prior" information about r_m and estimated r by fitting the Prager model to a composite index based on the 1981–1995 CPUE data. Our attempt to replicate this assessment using the same Prager model and catch and CPUE data produced an estimate of 0.7. Close analysis of detailed model output in NMFS (1996) indicates that there may have been a failure in numerical convergence. In contrast, Bayesian demographic methods produced a posterior median estimate of r of 0.07, a value consistent with demographic data on LCSs.

This latter estimate of r is preferable because it accounts for life history information that the NMFS (1996) estimate leaves out. This estimate is also consistent with estimates of r_0 for marine animals with similar reproductive life history characteristics (e.g., baleen whales (Best 1993)). Moreover, the CPUE data are not informative for r and, as also indicated by the very high estimate of 0.7, cannot be relied on by themselves to provide an accurate estimate of r.

It is thus important for stock assessments that use a Schaefer model to incorporate prior information on r, particularly when the time series data are noninformative with respect to r. As in the current example, time series data are uninformative often because they show a classic one-way trip (Hilborn and Walters 1992): taken together, the relative abundance data to which the production model is fitted conform only to a decreasing pattern over time. This is common in many fisheries, especially developing ones and heavily exploited ones (McAllister and Kirkwood 1998*b*). In order for relative abundance data to be informative about r, they must show at least one decreasing and one increasing pattern over time resulting from varying levels of exploitation intensity (Hilborn and Walters 1992; McAllister and Kirkwood

Fig. 7. Marginal posteriors for (*a*) *K*, (*b*) *r*, (*c*) N_{98}/K, and (*d*) MSY when the prior median for *r* was changed from 0.07 (solid line) to 0.11 (long-dashed line) and 0.20 (short-dashed line).

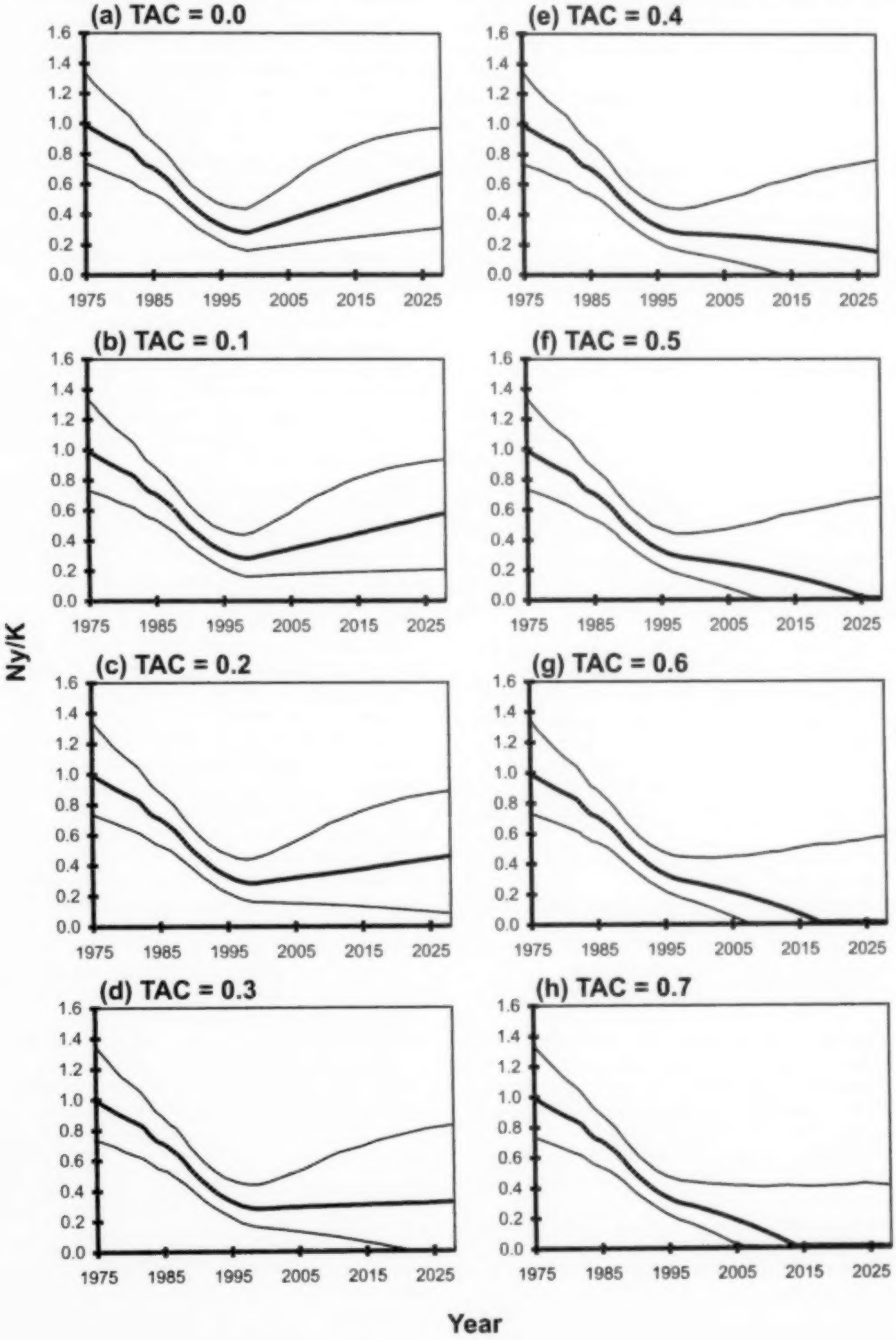


1998*a*). If they do not, then the resulting point estimate of *r* can be highly imprecise and possibly strongly biased, as in the present example. The joint posterior for *r* and *K* from a uniform prior for *r* shows that there is a large set of combinations of values for these parameters that fit the data almost equally well. In such cases, a prior for *r* based on demographic analysis or hierarchical methods (Myers et al. 1999) can be particularly useful for providing estimates of *r* consistent with knowledge about the organism's life history.

From a practical point of view, the use of such prior information is important because the values assumed for *r* can strongly determine the ability of a population to recover, if it has been heavily depleted. For example, NMFS (1996) applied the estimate for *r* of 0.26 in its evaluation of alternative recovery options. As this value for *r* appears to be too high, it is likely that the policy projections of the 1996 SEW are too optimistic. In contrast, the inclusion of demographic information about *r* should provide a more biologically consistent basis with which to evaluate alternative recovery options.

Bayesian estimation enabled the incorporation of other sensible assumptions about population dynamics. For example, an informative prior about the ratio of stock size in 1975 to carrying capacity was incorporated based on the information that fishing for sharks only started to pick up in the mid-1970s (J. Musick, Virginia Institute of Marine Science,

Fig. 8. Median values (thick line) and 90% probability intervals (thin lines) for stock size divided by K with alternative values for the TAC after 1998. The panels show the results under alternative constant TAC policies for fractions of the 1995 reported catch ranging from 0.0 to 0.7 of 367 200 fish. Results were produced with the baseline Bayesian assessment (e.g., prior median for $r = 0.07$).



College of William and Mary, School of Marine Science, Gloucester Point, VA 23062, U.S.A., personal communication). In order to account for the missing catch data between 1975 and 1980 and fit the model also to the CPUE data in these years, we also incorporated an informative prior for the mean catch between 1975 and 1980 based on the mean catches between 1981 and 1995.

As with other recent articles on Bayesian stock assessment (McAllister and Kirkwood 1998*a*, 1998*b*; Patterson 1999), this paper also demonstrates that the use of Bayesian decision analytic methods could help fishery managers to implement a deliberately precautionary approach (FAO 1995) to fishery management. The methods can be used to account for some highly influential uncertainties in population dynamics and provide a rational and objective means to evaluate the trade-offs of decreasing harvests and increasing the chances of achieving a stock recovery.

Acknowledgments

This work was made possible by a grant from the David and Lucile Packard Foundation and the Pew Charitable Trust. We thank Merry Camhi, Geoff Kirkwood, Luis Borda de Agua, Jack Musick, Gerry Scott, Joe Powers, Enric Cortes, Doug Butterworth, and Panayiota Apostolaki for helpful comments and discussions. Enric Cortes and an anonymous reviewer are thanked for their comments on the manuscript.

References

Adkison, M.D., and Peterman, R.M. 1996. Results of Bayesian methods depend on details of implementation: an example of estimating salmon escapement goals. Fish. Res. **25**: 155–170.

Anonymous. 1997. Atlantic shark fisheries; quotas, bag limits, prohibitions, and requirements. Rules and Regulations. Federal Register. Department of Commerce. NOAA Doc. 961211348-7065-03.

Begon, M., Harper, J.L., and Townsend, C.R. 1996. Ecology: individuals, populations and communities. 3rd ed. Blackwell Science, Oxford, U.K.

Berger, J.O. 1985. Statistical decision theory and Bayesian analysis. 2nd ed. Springer-Verlag, New York.

Best, P.B. 1993. Increase rates in severely depleted stocks of baleen whales. ICES J. Mar. Sci. **50**: 169–186.

Cortes, E. 1998. Demographic analysis as an aid in shark stock assessment and management. Fish. Res. **39**: 199–208.

Ebert, T.A. 1999. Methods in demography. Academic Press, San Diego, Calif.

Evans, M., Hastings, N., and Peacock, B. 1993. Statistical distributions. 2nd ed. John Wiley & Sons, New York.

Fisheries and Agriculture Organization of the United Nations (FAO). 1995. Part 1. Guidelines on the precautionary approach to capture fisheries. FAO Fish. Tech. Pap. 350/1.

Fisheries and Agriculture Organization of the United Nations (FAO). 2000. The state of world fisheries and aquaculture 2000. FAO Report. FAO, Rome.

Gelman, A., Carlin, J., Stern, H., and Rubin, D. 1995. Bayesian data analysis. Chapman and Hall, New York.

Hilborn, R., and Walters, C.J. 1992. Quantitative fisheries stock assessment: choice, dynamics, and uncertainty. Chapman and Hall, New York.

Hoenig, J.M., Warren, W.G., and Stocker, M. 1994. Bayesian and related approaches to fitting surplus production models. Can. J. Fish. Aquat. Sci. **51**: 1823–1831.

Kinas, P.G. 1996. Bayesian fishery stock assessment and decision making using adaptive importance sampling. Can. J. Fish. Aquat. Sci. **53**: 414–423.

Krebs, C.J. 1985. Ecology: the experimental analysis of distribution an abundance. 3rd ed. Harper and Row, New York.

Liermann, M., and Hilborn, R. 1997. Depensation in fish stocks: a hierarchic Bayesian meta-analysis. Can. J. Fish. Aquat. Sci. **54**: 1976–1984.

Lotka, A.J. 1907. Studies on the mode of growth of material aggregates. Am. J. Sci. **24**: 199–216.

McAllister, M.K., and Ianelli, J.N. 1997. Bayesian stock assessment using catch-age data and the sampling/importance resampling algorithm. Can. J. Fish. Aquat. Sci. **54**: 284–300.

McAllister, M.K., and Kirkwood, G.P. 1998*a*. Bayesian stock assessment: a review and example application using the logistic model. ICES J. Mar. Sci. **55**: 1031–1060.

McAllister, M.K., and Kirkwood, G.P. 1998*b*. Using Bayesian decision analysis to help achieve a precautionary approach to managing newly developing fisheries. Can. J. Fish. Aquat. Sci. **55**: 2642–2661.

McAllister, M.K., Pikitch, E.K., Punt, A.E., and Hilborn, R. 1994. A Bayesian approach to stock assessment and harvest decisions using the sampling/importance resampling algorithm. Can. J. Fish. Aquat. Sci. **51**: 2673–2687.

Meyer, R., and Millar, R.B. 1999. BUGS in Bayesian stock assessments. Can. J. Fish. Aquat. Sci. **56**: 1078–1086.

Millar, R.B., and Meyer, R. 2000. Non-linear state space modeling of fisheries biomass dynamics by using Hastings–Metropolis within-Gibbs sampling. Appl. Stat. **49**: 327–342.

Myers, R.A., Mertz, G., and Fowlow, P.S. 1997. Maximum population growth rates and recovery times for Atlantic cod, *Gadus morhua*. Fish. Bull. U.S. **95**: 762–772.

Myers, R.A., Bowen, K.G., and Barrowman, N.J. 1999. Maximum reproductive rate of fish at low population sizes. Can. J. Fish. Aquat. Sci. **56**: 2404–2419.

National Marine Fisheries Service (NMFS). 1996. Report of the Shark Evaluation Workshop. National Oceanic and Atmospheric Association, NMFS, Southeast Fisheries Science Center, 75 Virginia Beach Dr., Miami, FL 33149, U.S.A.

National Marine Fisheries Service (NMFS). 1997. Report of the Shark Evaluation Workshop. National Oceanic and Atmospheric Association. National Oceanic and Atmospheric Association, NMFS, Southeast Fisheries Science Center, 75 Virginia Beach Dr., Miami, FL 33149, U.S.A.

National Marine Fisheries Service (NMFS). 1998. Report of the Shark Evaluation Workshop. National Oceanic and Atmospheric Association, NMFS, Southeast Fisheries Science Center, 3500 Delwood Beach Rd., Panama City, FL 32408, U.S.A.

Patterson, K.R. 1999. Evaluating uncertainty in harvest control law catches using Bayesian Markov chain Monte Carlo virtual population analysis with adaptive rejection sampling and including structural uncertainty. Can. J. Fish. Aquat. Sci. **56**: 208–221.

Prager, M.H. 1994. A suite of extensions to a nonequilibrium surplus production model. Fish. Bull. U.S. **92**: 374–389.

Punt, A.E., and Hilborn, R. 1997. Fisheries stock assessment and decision analysis: the Bayesian approach. Rev. Fish Biol. Fish. **7**: 35–63.

Quinn, T.J., II, and Deriso, R.B. 1999. Quantitative fish dynamics. Oxford University Press, New York.

Rubin, D.B. 1988. Using the SIR algorithm to simulate posterior distributions. *In* Bayesian Statistics 3: Proceedings of the Third Valencia International Meeting, June 1–5, 1987. *Edited by* J.M. Bernardo, M.H. Degroot, D.V. Lindley, and A.M. Smith. Clarendon Press, Oxford, U.K. pp. 385–402.

Smith, S.E., Au, D., and Shaw, C. 1998. Intrinsic rebound potentials of 26 species of Pacific sharks. Mar. Freshwater Res. **49**: 663–678.

Walters, C.J., and Ludwig, D. 1994. Calculation of Bayes posterior probability distributions for key population parameters: a simplified approach. Can. J. Fish. Aquat. Sci. **51**: 713–722.

Appendix A. *r* in numbers or biomass?

This appendix proves that under the assumption of stationary weight at age, r is independent of population abundance in numbers or biomass. In a biomass dynamic model, r has the following interpretation:

$$\text{(A1)} \quad B_{t+1} = \lim_{B_t \to 0} B_t \exp(r)$$

where B_t is population biomass in year t. Under such conditions and stable age structure, r can be obtained from

$$\text{(A2)} \quad r = \lim_{B_t \to 0} \log\left(\frac{B_{t+1}}{B_t}\right)$$

or

$$\text{(A3)} \quad r = \lim_{B_t \to 0} \log\left(\frac{\sum_{x=0}^{A} w_{x,t+1} N_{x,t+1}}{\sum_{x=0}^{A} w_{x,t} N_{x,t}}\right)$$

where $w_{x,t}$ and $N_{x,t}$ are the weight and abundance at age x in year t. This proof assumes that at very low population density, the weight at age is likely to be unaffected by intraspecific competition. Therefore, the vectors of weight at age in time intervals t and $t + 1$ are assumed to be the same between time steps.

If weight at age is constant at low population density, then the intrinsic rate of increase for a model in biomass is the same as that for a model in abundance. We demonstrate this as follows. First, the expression for r in abundance can be expressed as

$$\text{(A4)} \quad r = \lim_{N_t \to 0} \log\left(\frac{\sum_{x=0}^{A} N_{x,t+1}}{\sum_{x=0}^{A} N_{x,t}}\right)$$

Since this equation holds only if the age structure is stable, the expression can be rewritten as

$$\text{(A5)} \quad r = \lim_{N_t \to 0} \log\left(\frac{H_{t+1} \sum_{x=0}^{A} c_x}{H_t \sum_{x=0}^{A} c_x}\right)$$

where c_x is the proportion of individuals of age x and $H_t c_x = N_{t,x}$. This expression can be simplified as follows:

$$\text{(A6)} \quad r = \lim_{N_t \to 0} \log\left(\frac{H_{t+1}}{H_t}\right)$$

or

$$\text{(A7)} \quad r = \lim_{N_t \to 0} \log(Q)$$

where Q is a constant. This same result can be obtained for a model in biomass:

$$\text{(A8)} \quad r = \lim_{B_t \to 0} \log\left(\frac{\sum_{x=0}^{A} w_{x,t+1} N_{x,t+1}}{\sum_{x=0}^{A} w_{x,t} N_{x,t}}\right)$$

and from eq. A5:

$$\text{(A9)} \quad r = \lim_{B_t \to 0} \log\left(\frac{H_{t+1}\sum_{x=0}^{A} w_x c_x}{H_t \sum_{x=0}^{A} w_x c_x}\right)$$

Simplifying:

$$\text{(A10)} \quad r = \lim_{B_{t\to 0}} \log\left(\frac{H_{t+1}}{H_t}\right)$$

and from eqs. A6 and A7:

$$\text{(A11)} \quad r = \lim_{B_{t\to 0}} \log(Q)$$

This is precisely the same result obtained with the equations in abundance (eq. A7). Therefore, r is the same for a model in abundance or biomass. The r is directly related to somatic growth because there is often a strong relationship between individual growth rate, the age at maturity, and fecundity at age. The effect of somatic growth on r is manifested in the maturity and fecundity at age schedule. The demographic methods for computing r_m thus implicitly incorporate somatic growth rates and apply equally well to Schaefer models in biomass and abundance. Application of matrix algebra to the Leslie matrix yields the same dominant eigenvalue and value for r with and without the weight at age vector, providing that this vector remains constant.

Appendix B. *r* for exploited or mature animals?

This appendix demonstrates that r is independent of the age groupings considered in the population modeled in a Schaefer model. Using an expression for r^{i+} for the abundance of age i+ individuals, r^{i+} can be equated with

$$\text{(B1)} \quad r^{i+} = \lim_{N_t^{i+} \to 0} \log\left(\frac{\sum_{x=i}^{A} N_{x,t+1}}{\sum_{x=i}^{A} N_{x,t}}\right)$$

where $0 \le i \le$ A. Using $N_{x,t} = H_t c_x$ where c_x is the "stabilized" proportion of individuals of age x (i.e., if the Leslie population matrix was projected indefinitely):

$$\text{(B2)} \quad r^{i+} = \lim_{N_t^{i+} \to 0} \log\left(\frac{H_{t+1}\sum_{x=i}^{A} c_x}{H_t \sum_{x=i}^{A} c_x}\right)$$

or

$$\text{(B3)} \quad r^{i+} = \lim_{N_t^{i+} \to 0} \log(Q)$$

where again:

$$\text{(B4)} \quad Q = \frac{H_{t+1}}{H_t}$$

Thus, r is independent of the age-classes included and modeled in the quantity P_t. The same can be shown for the biomass quantity B_t. The same can also be shown if recruitment to P_t is not knife-edged at age i but occurs over a number of age-classes. Therefore, demographic methods to estimate r are also independent of the age or pattern of recruitment to the modeled population.

Surplus production, variability, and climate change in the great sardine and anchovy fisheries

**Larry D. Jacobson, Jose A.A. De Oliveira, Manuel Barange,
Miguel A. Cisneros-Mata, Roberto Félix-Uraga, John R. Hunter, Jin Yeong Kim,
Yasunobu Matsuura, Miguel Ñiquen, Carmela Porteiro, Brian Rothschild,
Ramiro P. Sanchez, Rodolfo Serra, Andres Uriarte, and Tokio Wada**

Abstract: We used fishery and survey data to calculate annual surplus production (ASP) and instantaneous surplus production rates (ISPR) for eight anchovy and nine sardine stocks. In addition, we calculated ASP per unit spawning area for six anchovy and six sardine stocks. Median ASP was highest for stocks with highest median biomass (mostly anchovies), and ASP was typically about 16% of stock biomass. ASP was often negative, more frequently for anchovies (36% of years) than for sardines (17% of years). ISPR was less variable for sardines and autocorrelated for longer-lived stocks (mostly sardines). Strong biomass increases tended to be preceded by short, abrupt increases in ISPR, and declines were pronounced when catches exceeded ASP for 5 years or more. The longest "runs" of positive and negative production were 21 and 4 years for sardine off Japan, 10 and 3 years for sardine off California, 8 and 2 years for anchovy off Peru, and 4 and 3 years for anchovy off California. ISPR is more sensitive to environmental changes than catch, biomass, or ASP and appear to be better for identifying environmentally induced regime shifts. Long time series show evidence of density-dependent effects on ASP in anchovies and sardines, but environmentally induced variation appears to dominate.

Résumé : Des statistiques de pêche et des données d'inventaire nous ont servi à calculer la production excédentaire annuelle (ASP) et les taux instantanés de production excédentaire (ISPR) chez huit stocks d'anchois et neuf stocks de sardines. De plus, nous avons calculé l'ASP par unité de surface de frayère chez six stocks d'anchois et six stocks de sardines. L'ASP moyen est maximal chez les stocks qui possèdent la plus grande biomasse médiane (surtout des anchois) et correspond normalement à environ 16% de la biomasse du stock. L'ASP est souvent négative, plus souvent chez les anchois (36% des années) que chez les sardines (17% des années). L'ISPR est moins variable chez les sardines et est autocorrelé chez les stocks à longévité plus grande (surtout des sardines). Les augmentations importantes de biomasse sont normalement précédées d'une croissance abrupte de l'ISPR; lorsque les prises dépassent l'ASP pour

Received January 31, 2001. Accepted April 25, 2001. Published on the NRC Research Press Web site at http://cjfas.nrc.ca on August 30, 2001.
J16200

L.D. Jacobson[1,2] **and J.R. Hunter.** National Marine Fisheries Service, Southwest Fisheries Science Center, P.O. Box 271, La Jolla, CA 92038, U.S.A.
J.A.A. De Oliveira. Marine and Coastal Management, Private Bag X2, 8012 Rogge Bay, South Africa.
M. Barange. GLOBEC International Project Office, Plymouth Marine Laboratory, Prospect Place, Plymouth PL1 3DH, U.K.
M.A. Cisneros-Mata. Instituto Nacional de la Pesca, Centro Regional de Investigaciones Pesqueras (INP-CRIP), Calle 20 No. 605 Sur, C.P. 85400, Guaymas, Sonora, Mexico.
R. Félix-Uraga. Centro Interdisciplinario de Ciencias Marinas (CICIMAR), Instituto Politecnico Nacional, Playa El Conchalito S/N, Apartado Postal 592, C.P. 23096, La Paz, B.C.S., Mexico.
J.Y. Kim. National Fisheries Research and Development Institute, West Fisheries Research Institute, No. 98-36, 1Ga, Bukseong-Dong, Jung-Gu, Incheon, 400-201 South Korea.
Y. Matsuura. Instituto Oceanografico, Universidade de Sao Paulo, Butantan, Sao Paulo, 05508, Brazil.
M. Ñiquen. Instituto del Mar del Peru (IMARPE), Esquina Gamarra y General Valle, Apdo. Postal 22, Chucuito, Callao, Peru.
C. Porteiro. Instituto Español de Oceanografia, Centro Oceanográfico de Vigo, Apartado de Correos 1552, Cabo Estay Canido, Vigo 36280, Spain.
B. Rothschild. School of Marine Science and Technology, University of Massachusetts, 706 South Rodney French Boulevard, New Bedford, MA 02744-1221, U.S.A.
R.P. Sanchez. Instituto Nacional de Investigación y Desarrollo Pesquero, P.O. Box 175, 7600 Mar del Plata, Argentina.
R. Serra. Instituto de Fomento Pesquero, Casilla 8-V, Valparaiso, Chile.
A. Uriarte. Instituto Tecnológico Pesquero y Alimentario (AZTI), Avda. Satrustegui, 8, San Sebastián, 20008, Spain.
T. Wada. National Research Institute of Fisheries Science, Japan Ministry of Agriculture, Forestry and Fisheries, 2-12-4 Fukuura, Kanazawa-ku, Yokohoma, Kanagawa-ken 236, Japan.

[1] Corresponding author (e-mail: larry.jacobson@noaa.gov).

[2] Present address: National Marine Fisheries Service, Northeast Fisheries Science Center, 166 Water Street, Woods Hole, MA 02543-1026, U.S.A.

Can. J. Fish. Aquat. Sci. **58**: 1891–1903 (2001) DOI: 10.1139/cjfas-58-9-1891

5 ans ou plus, il y a un déclin prononcé. Les plus longues « séquences » de production positive et négative ont été respectivement de 21 et 4 années pour les sardines au large du Japon, de 10 et 3 années pour les sardines au large de la Californie, de 8 et 2 années pour les anchois au large du Pérou et de 4 et 3 années pour les anchois au large de la Californie. Les ISPR est plus sensible aux changements de l'environnement que ne le sont les captures, la biomasse ou l'ASP et semblent être plus appropriés pour détecter les changements de régime dus à l'environnement. Les longues séries temporelles laissent croire à l'existence d'effets de densité-dépendance sur l'ASP chez les anchois et les sardines, mais la variation attribuable à l'environnement semble dominer.

[Traduit par la Rédaction]

Introduction

Annual surplus production (ASP) is biological production in excess of the amount required, when no fishing occurs, to maintain the same stock biomass level at the beginning of consecutive years (Ricker 1975). ASP is the net result of recruitment, somatic growth, and mortality. It can be expressed as the product of stock biomass and the rate of ASP per unit biomass. The rate may be density independent (constant over time or variable due to environmental factors only), density dependent (variable due to stock biomass), or both.

ASP is a fundamental variable in fishery science and fisheries management. Maximum sustained yield (MSY) is proportional to maximum ASP (i.e., $\bar{\delta}\text{MSY} = \text{ASP}_{\text{max}}$, see below). In any year, catches larger than $\text{ASP}/\bar{\delta}$ reduce stock biomass, while smaller catches increase stock biomass. If ASP is negative, then stock biomass will decline, even with no catch. Thus, ASP is important when managers seek to optimize economic benefits from a fishery, adjust harvest levels to attain biomass targets, prevent overfishing, or rebuild overfished stocks.

According to the theory of density dependence (e.g., Schaefer 1957), and neglecting environmental effects, fishing reduces stock biomass but increases the rate of production per unit biomass because of reduced competition for food and habitat, removal of older, slow-growing individuals, and other factors. If the increased rate of production exceeds the proportional reduction in biomass, then ASP for the stock as a whole increases, and vice versa. ASP is zero at the theoretical extremes when biomass is zero (but the production rate is maximum) and at carrying capacity when stock biomass is high (but the production rate is zero). In theory, maximum ASP occurs at an intermediate biomass level that depends on the shape of the spawner–recruit relationship, density-dependent growth and mortality, fishing mortality rates by age and size, and other factors (Sissenwine and Shepherd 1987). Density-dependent effects on ASP are important because fishing reduces stock biomass and keeps the engine of production "turned on." Wada and Jacobson (1998) suggested that density-dependent effects on recruitment (an important component of ASP) are typical in sardines but difficult to measure without many years of data and models that accommodate environmental variation.

In contrast with density-dependent effects, environmental effects on ASP by small pelagic fish like sardines and anchovies are often more dramatic, even in short time series (e.g., Barnes et al. 1992), because most small pelagic fish are short-lived and planktivorous (Hunter and Alheit 1995).

Small pelagic fish are major components of large marine ecosystems where they may affect the population dynamics of their planktivorous prey and predators (Cury et al. 2000), which include marine mammals, birds, and other fish (Hunter and Alheit 1995). Small pelagic species provide a large fraction of the total global catch of marine fish and support some of the largest individual fisheries in the world (Hunter and Alheit 1995). Dramatic changes in ASP and biomass due to recruitment variability, rapid somatic growth, and high mortality rates are characteristic of small pelagic fish, particularly with fishing. Thus, ASP is useful in the study of responses of anchovy and sardine stocks to environmental change, such as regime shifts. We define regimes as periods of high and low surplus production rates (see below) associated with decadal-scale variability in the environment (Trenberth 1990; Lluch-Belda et al. 1992; Schwartzlose et al. 1999).

The main purpose of this paper is to measure and characterize variability in ASP and ASP rates for anchovy (*Engraulis* spp.) and sardine stocks (*Sardinops*, *Sardinella*, and *Sardina* spp.) around the world and to evaluate them as a way to detect regime shifts (Lluch-Belda et al. 1989, 1992). We hypothesize that ASP rates are more sensitive than other population indicators and therefore more effective in identifying regime shifts. Our research was part of a plan coordinated by the Small Pelagic Fish and Climate Change (SPACC) Program of the Global Ocean Ecosystem Dynamics (GLOBEC) Program, with the ultimate objective of understanding and predicting climate-induced changes in the production of small pelagic fish around the world.

Materials and methods

ASP was calculated for annual periods (years, usually calendar years or July–June fishing years) used by managers in assessments for each stock. We restricted analyses to fish ages 1 and older (ages 1+) because anchovies and sardines spawn and recruit throughout the year (Blaxter and Hunter 1982) and recruitment is complete by age 1. Our calculations exclude ASP by age-0 fish (see below). All production calculations and biomass and catch data are in units of body weight.

Catch data used in this analysis were official estimates of total catch in weight (all ages) less an estimate of the catch weight of age-0 fish (Table 1). Age-0 fish (young of the current year) are typically less than 10% of the total catch weight in most anchovy and sardine fisheries (Table 1). It was not always possible to deduct the catch weight of age-0 fish from official catch estimates, however, due to data limitations. The relative error in using catch data that include age-0 fish is a positive bias proportional to catch weight of age-0 fish and inversely proportional to production by older fish (Appendix A).

As described above, ASP is defined as the change in stock biomass from one year to the next with no fishing. ASP by a single cohort (age group) in an unfished stock is

$$P_{t,a} = B^*_{t+1,a+1} - B_{t,a} \quad (1)$$

where $B_{t,a}$ is the biomass of the cohort at age a and at the beginning of year t. The asterisk is for the special case of no fishing during year t. ASP by a cohort in a fished stock depends on catch as well as biomass:

$$(2)\qquad P_{t,a} = B_{t+1,a+1} - B_{t,a} + \delta_{t,a} C_{t,a}$$

where $C_{t,a}$ is catch weight and $\delta_{t,a}$ converts catches to units of equivalent population biomass at the beginning of the next year (MacCall 1978; Opsomer and Conrad 1994) to account for losses and gains due to natural mortality and growth that would have occurred if the catch had not been taken. For simplicity, it is assumed that the fishery operates continuously throughout the year (MacCall 1978), although most anchovy and sardine fisheries are seasonal.

In some cases, age-specific estimates of abundance and catch were not available (e.g., Jacobson et al. 1994). We therefore modified eq. 2 to approximate ASP for ages 1+:

$$(3)\qquad P_t = B_{t+1} - B_t + \bar{\delta} C_t$$

where $\bar{\delta}$ was an average (see below) adjustment factor, assumed constant over time. We considered using time-dependent catch adjustment factors but found too much variability in annual growth data and mortality estimates. In our calculations, recruitment at the beginning of year $t + 1$ (part of B_{t+1}) was counted as production during year t.

The adjustment factor $\bar{\delta}$ was an approximate weighted average:

$$(4)\qquad \bar{\delta} = \frac{\sum_{a=1}^{A} \tilde{C}_a \delta_a}{\sum_{a=1}^{A} \tilde{C}_a}$$

where $\tilde{C}_a$ was proportional to age-specific catch weight per recruit at average fishing mortality rates (see below). Based on a quadratic regression model for the results in table 1 of MacCall (1978) (with $F = 0.5\cdot\text{year}^{-1}$), age-specific adjustment factors were, $\delta_a = 0.999 - 0.529k_a + 0.0899k_a^2$ where $K_a = M - G_a$ was the difference between instantaneous rates for natural mortality (M) and growth ($\bar{G}_a$) (see below).

Average instantaneous growth rates were $\bar{G}_a = \ln(w_{a+1}/w_a)$ with weights at age (w_a) from a von Bertalanffy curve (Ricker 1975). We used a single von Bertalanffy growth curve to smooth the data and obtain approximate average growth rates.

The term used to compute $\bar{\delta}$ in eq. 4 was

$$(5)\qquad \tilde{C}_a = \frac{\bar{F}_a}{\bar{Z}_a}\left(1 - e^{-\bar{Z}_a}\right)\beta_a$$

where $\bar{Z}_a = \bar{F}_a + M - \bar{G}_a$ and $\bar{F}_a$ was the average fishing mortality rate for age a from stock assessment work (constant for all ages when age-specific estimates were not available). In our calculations, $\tilde{C}_a$ was approximately proportional to age-specific catch weight per recruit at average fishing mortality rates because

$$\beta_a = e^{\sum_{t=0}^{a-1} -\bar{Z}_t} \quad (\text{with } \bar{Z}_0 = 0)$$

was proportional to age-specific biomass per recruit.

Summary statistics

To make comparisons among stocks and species, we calculated ASP (in kilotonnes per year), as described above, as well as the instantaneous surplus production rate (ISPR):

$$(6)\qquad \rho_t = \ln\left[\frac{P_t + B_t}{B_t}\right]$$

with units per year. ISPR measures the net rate of somatic growth, recruitment, and natural mortality. With no fishing in year t, $B^*_{t+1} = B_t e^{\rho_t}$; with fishing, $B_{t+1} = B_t e^{\rho_t - F_t}$ where F_t is the instantaneous fishing mortality rate (Appendix B summarizes the relationships among ρ_t, F_t, and C_t). In either case, ASP is $P_t = B_t(e^{\rho_t} - 1)$.

ISPR calculations for anchovies and sardines are useful because they help distinguish between effects on ASP due to changes in stock biomass B_t and changes in the rate of production per unit biomass (measured by ρ_t). That is, ISPR calculations convert estimates of gross ASP (which vary among stocks due to overall stock size) to instantaneous "per capita" measures that can, like instantaneous fishing mortality rates, be compared for stocks of different size. The logarithm in eq. 6 makes distributions of annual instantaneous rates more symmetrical and easier to compare. Other measures, such as production per unit biomass (P_t/B_t), are similar to ISPR. We also calculated production per unit spawning area $\varphi_t = P_t/A_t$ when estimates of spawning area (A_t) were available.

To minimize the effects of measurement and estimation errors in biomass estimates (often considerable, e.g., Jacobson et al. 1994; Deriso et al. 1996), we used medians ($Q_{50\%}$) to measure the central tendency of estimates. We used 10% and 90% quantiles ($Q_{10\%}$ and $Q_{90\%}$) to measure range and the difference between the median and 84% quantile $\sigma_r = (Q_{84\%} - Q_{50\%})$ as a "robust" measure of standard deviation. The robust statistic was analogous to traditional standard deviation values because, for the normal distribution, $Q_{84\%}$ is 1 SD to the right of the mean.

Data

Biomass estimates were available for eight anchovy stocks (*Engraulis* spp.) (Table 1). Catch data were available for all anchovy stocks with the exception of two almost unexploited stocks off Northern Patagonia and Uruguay – Buenos Aires (Table 1). Biomass and catch data were available for nine exploited sardine stocks (*Sardinops* spp., *Sardinella brasiliensis*, and *Sardina pilchardus*) (Table 1).

We used the best available information for each stock in surplus production calculations. Depending on advice from the responsible scientists, the type of biomass estimate, and situation, we omitted up to five of the most recent biomass estimates because recent estimates are often the least reliable (Pope 1972). When necessary, official biomass statistics for ages 0+ were adjusted by subtracting the biomass of age-0 fish. Biomass estimates for *S. sagax* off northern Chile and southern Peru began at age 2, and we back-calculated biomass of younger age groups assuming natural mortality with no fishing. As described above, catch weights of age-0 fish were deducted from official catch records where possible (Table 1).

Results

Natural mortality rates (M) assumed in stock assessment work for stocks in our study ranged from 0.8 to $1.2\cdot\text{year}^{-1}$ (median $1.1\cdot\text{year}^{-1}$) for anchovies and from 0.3 to $0.96\cdot\text{year}^{1}$ (median $0.51\cdot\text{year}^{1}$) for sardines (Table 1). For most stocks, average adjustment factors for catch ($\bar{\delta}$) were large enough to affect production calculations. They ranged from 0.61 to 0.78 (median 0.67) for anchovies and from 0.76 to 1.03 (median 0.85) for sardines (Table 1).

Natural mortality rates and average adjustment factors for catch data were highly correlated (Fig. 1). This could be ex-

Table 1. Anchovy and sardine stocks and stock areas, years with biomass estimates (at the time of the analysis), years with production estimates used in this study, natural mortality rates (*M*) from stock assessments, average catch adjustment factors ($\bar{\delta}$), and average percentage (by weight) of age-0 fish in catches.

Stock and stock area	Years with biomass estimates (first year–last year)	Years with production estimates (first year–last year (*N*))	*M* (year^{-1})	$\bar{\delta}$	Age-0 fish in catch
Anchovies					
Engraulis anchoita (N. Patagonia)[a]	1993–1995	1993–1994 (2)	1.1	na	na
E. anchoita (Uruguay – Buenos Aires)[a]	1993–1995	1993–1994 (2)	1.1	na	na
E. capensis (South Africa)[b]	1985–1995	1985–1994 (10)	1.2	0.62	Substantial; no adjustments
E. encrasicolus (Bay of Biscay)[c]	1987–1995	1987–1993 (7)	1.2	0.61	Range 1–22%; mean 7%; adjusted
E. japonica (Japan)[d,f]	1978–1997	1978–1996 (19)	Mean 1.1	0.77	Range 2–40%; mean 15%; adjusted
E. mordax (California)[e]	1953–1995	1953–1990 (38)	0.8	0.71	Mean probably <10%; no adjustments
E. ringens (N. Chile – S. Peru)[f]	1984–1993	1984–1990 (7)	1.0	0.61	range 0–12%; mean 4%; adjusted
E. ringens (Peru)[f]	1962–1993	1962–1989 (28)	Mean 0.92	0.78	Range 0–33%; mean 8%; adjusted
Sardines					
Sardinops melanostictus (Japan)[g]	1951–1995	1951–1991 (41)	0.4	0.92	Range 0–20%; mean 5% during 1978–1999; pre-1978 unknown; no adjustments
S. melanostictus (South Korea)[f]	1971–1996	1971–1992 (22)	0.51	0.85	Range 0–22%; mean 3%; adjusted
S. sagax (South Africa)[f]	1988–1996	1988–1993 (6)	0.6	0.85	Substantial; no adjustments
S. sagax (N. Chile – S. Peru)[f]	1974–1993	1974–1988 (15)	0.3	0.94	Zero; no adjustments
S. sagax (California)[h]	1932–1996	1932–1961, 1983–1992 (40)	0.4	0.83	Mean probably <10%; no adjustments
S. sagax (Gulf of California)[i]	1972–1989	1972–1984 (13)	0.7	0.86	Range 0–3%; mean 1%; no adjustments
S. sagax (Magdalena Bay)[j]	1981–1996	1981–1992 (12)	0.7	0.76	Range 0–28%; mean 5%; adjusted
Sardina pilchardus (Iberian Peninsula)[f]	1976–1996	1976–1994 (19)	0.33	1.03	Zero; no adjustments
Sardinella brasiliensis (Brazil)[k]	1977–1992	1977–1988 (12)	0.96	0.71	<1%; no adjustments

Note: na, not applicable.

[a] Sánchez et al. (1996).

[b] Butterworth et al. (1993).

[c] International Council for the Exploration of the Sea (1996).

[d] Hayashi (1961).

[e] Jacobson et al. (1994), Jacobson et al. (1995), and Methot (1989).

[f] Unpublished estimates (Japanese data from T. Wada, NRIFS, Japan; Chile and Peru data from R. Serra, IFOP, Chile; Korea data from J.Y. Kim, NFRDI, South Korea; South Africa data from J. De Oliveira, MCM, South Africa; Iberian Peninsula data from A. Uriarte, AZTI, Spain).

[g] Wada and Jacobson (1998).

[h] Barnes et al. (1997) and Deriso et al. (1996).

[i] Cisneros-Mata et al. (1995).

[j] Félix-Uraga et al. (1996).

[k] Cergole (1995).

Fig. 1. Average catch adjustment factors ($\bar{\delta}$) used in production calculations and natural mortality rates used in stock assessments for sardines (open squares) and anchovies (solid circles).

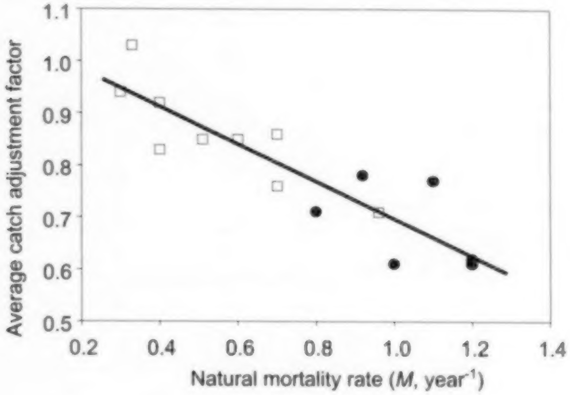


Fig. 2. Median ASP and median stock biomass for anchovy (solid circles) and sardine (open squares) stocks. Median production values near 2000 kt are for *E. encrasicolus* (northern Patagonia stock) and *E. capensis* off South Africa.

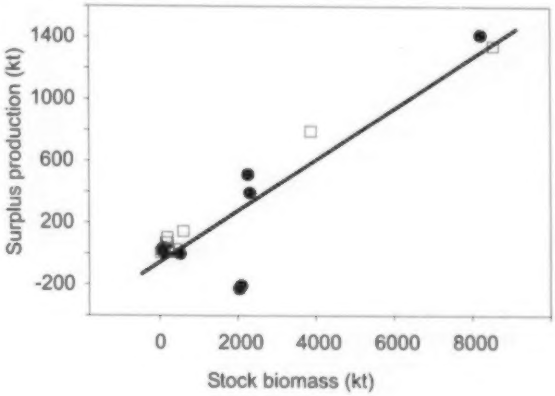


Table 2. Median biomass and median production estimates for anchovy and sardine stocks.

Stock and stock area	Median biomass (kt)	Median production (kt)
Anchovies		
Engraulis anchoita (N. Patagonia)	2105	–211
E. anchoita (Uruguay – Buenos Aires)	2339	390
E. capensis (South Africa)	2050	–230
E. encrasicolus (Bay of Biscay)	94	9
E. japanica (Japan)	91	34
E. mordax (California)	547	–1
E. ringens (N. Chile – S. Peru)	2274	507
E. ringens (Peru)	8238	1412
Sardines		
Sardinops melanostictus (Japan)	237	12
S. melanostictus (South Korea)	3880	794
S. sagax (South Africa)	172	74
S. sagax (N. Chile – S. Peru)	8549	1346
S. sagax (California)	224	21
S. sagax (Gulf of California)	202	106
S. sagax (Magdalena Bay)	41	7
Sardina pilchardus (Iberian Peninsula)	617	146
Sardinella brasiliensis (Brazil)	412	31

pected because age-specific adjustment factors (δ_a in eq. 4) were computed as a function of natural mortality rate (see above). However, based on our results, the linear regression model $\bar{\delta} = 1.05 - 0.353M$ ($R^2 = 78\%$, $n = 15$) would suffice to approximate average correction factors $\bar{\delta}$ for any sardine or anchovy stock.

Median ASP was highest for stocks with high median biomass, although South African and northern Patagonian anchovy stocks had relatively low median ASP levels (Table 2; Fig. 2). The correlation between median ASP and median biomass was 0.91. A linear regression equation $P_{\text{median}} = 0.16B_{\text{median}}$ (forced through the origin) suggests that ASP is typically about 16% of biomass in exploited anchovy and sardine stocks. Stocks with the highest biomass were mostly anchovies. The relatively low median ASP for South African anchovy was due to a declining biomass trend during 1985–1994, and the median ASP for the northern Patagonian anchovy stock was based on only 3 years of biomass estimates (Table 1). Larger sample sizes would likely give higher estimates of median ASP for both stocks.

Years of negative ISPR and ASP were common, particularly for anchovies, reflecting the possibility of declines in biomass, even in the absence of fishing. ISPR (and ASP) were negative in 36% of years ($n = 113$ for all years and all stocks) for anchovies and 17% of years ($n = 180$) for sardines (Table 3). For individual anchovy and sardine stocks, the percentage of years with negative ASP and ISPR ranged from 14 to 60% for anchovies and from 0 to 33% for sardines.

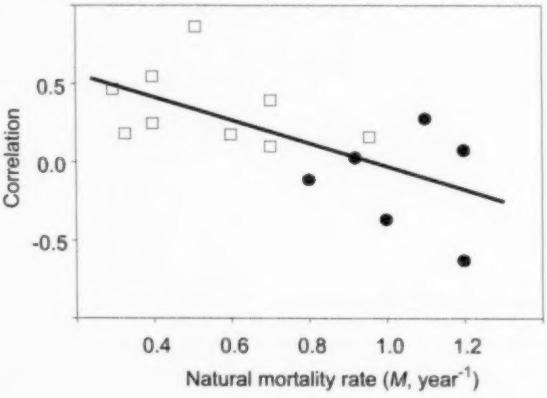
For anchovies, median ISPR (all years and all stocks) was 0.14 and the robust measure of standard deviation was σ_r = 0.52 (Table 3). For sardines, median ISPR was 0.18 and σ_r = 0.28. Production rates in consecutive years (Table 3; Fig. 3) were positively correlated for long-lived stocks with low nat-

Table 3. ISPR (year^{-1}) summary statistics.

Stock and stock area	$Q_{10\%}$	Median	$Q_{90\%}$	$Q_{90\%} - Q_{10\%}$	σ_r	% negative years	Lag 1 auto correlation
Anchovies							
Engraulis anchoita (N. Patagonia)	na	–0.11	na	na	na	50	na
E. anchoita (Uruguay – Buenos Aires)	na	0.20	na	na	na	50	na
E. capensis (South Africa)	–0.45	–0.07	0.64	1.09	0.42	60	0.07
E. encrasicolus (Bay of Biscay)	–0.20	0.16	0.88	1.09	0.67	43	–0.63
E. japanica (Japan)	–0.01	0.41	0.80	0.81	0.74	16	0.27
E. mordax (California)	–0.41	0.01	0.76	1.17	0.68	50	–0.12
E. ringens (N. Chile – S. Peru)	0.06	0.27	0.67	0.60	0.66	14	–0.37
E. ringens (Peru)	–0.24	0.17	0.66	0.90	0.48	25	0.02
All anchovies	–0.37	0.14	0.75	1.12	0.52	36	na
Sardines							
Sardinops melanostictus (Japan)	–0.30	0.10	0.49	0.79	0.48	27	0.54
S. melanostictus (South Korea)	0.09	0.21	0.42	0.34	0.41	0	0.85
S. sagax (South Africa)	0.11	0.43	0.66	0.55	0.58	0	0.17
S. sagax (N. Chile – S. Peru)	0.07	0.16	0.24	0.17	0.24	0	0.46
S. sagax (California)	–0.15	0.18	0.59	0.74	0.46	33	0.23
S. sagax (Gulf of California)	0.12	0.45	0.80	0.68	0.66	0	0.38
S. sagax (Magdalena Bay)	–0.09	0.12	0.63	0.72	0.50	25	0.09
Sardina pilchardus (Iberian Peninsula)	0.12	0.22	0.30	0.18	0.29	0	0.17
Sardinella brasiliensis (Brazil)	–0.06	0.08	0.37	0.43	0.28	33	0.16
All sardines	–0.08	0.18	0.51	0.59	0.28	17	na

Note: $Q_{10\%}$ and $Q_{90\%}$ are the 10 and 90% quantiles and σ_r is a robust measure of standard deviation. na, not applicable.

Fig. 3. Autocorrelation (lag 1 year) in ISPR for anchovies (solid circles) and sardines (open squares).

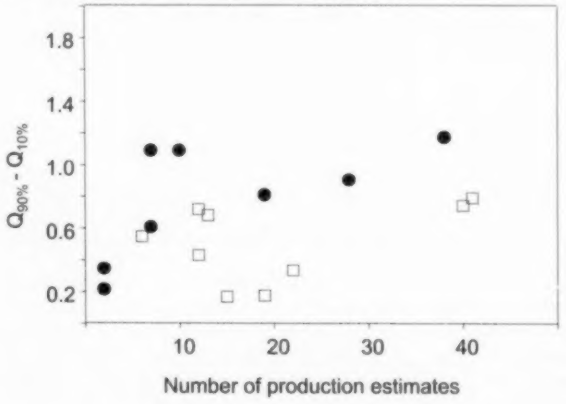


ural mortality rates (mostly sardines) and uncorrelated or negatively correlated for shorter-lived stocks with high natural mortality rates (mostly anchovies).

Long time series

About 30 years of data may be required to measure the full range in ISPR for anchovy and sardine stocks. The $Q_{90\%} - Q_{10\%}$ values (a measure of range, see above) for two sardine stocks (*S. melanostictus* off Japan and *S. sagax* off California) and two anchovy stocks (*E. ringens* off Peru and *E. mordax* off California) with at least 28 years of production data were generally higher than $Q_{90\%} - Q_{10\%}$ values for stocks with fewer years of production data (Tables 1 and 3; Fig. 4). However, this generalization did not hold true for two anchovy stocks (*E. encrasicolus* in the Bay of Biscay with 8 years of production data and *E. capensis* off South Africa with 10 years of production data). In the exceptions, high values of $Q_{90\%} - Q_{10\%}$ were observed despite relatively low numbers of years with production estimates.

Fig. 4. $Q_{90\%} - Q_{10\%}$ (a measure of range) for ISPR and number of years of production data for sardines (open squares) and anchovies (solid circles). The two points with high $Q_{90\%} - Q_{10\%}$ values at 8 and 10 years of data are for *E. encrasicolus* (Bay of Biscay) and *E. capensis* (South Africa). Data for two *E. anchoita* stocks with only 2 years of production data are excluded.



Stocks with at least 28 years of ASP estimates included two anchovy stocks and two sardine stocks. There were 38 years of ASP estimates for *E. mordax* off California, 28 years for *E. ringens* off Peru, 41 years for *S. melanostictus* off Japan, and 40 years for *S. sagax* off California. We ana-

Fig. 5. Left panels: catch data (open circles), biomass (solid circles), and ASP (solid triangles) estimates. Right panels: ISPR estimates for (*a* and *b*) Japanese sardine *S. melanostictus*, (*c* and *d*) Californian sardine *S. sagax*, (*e* and *f*) Peruvian anchovy *E. ringens*, and (*g* and *h*) Californian anchovy *E. mordax*.

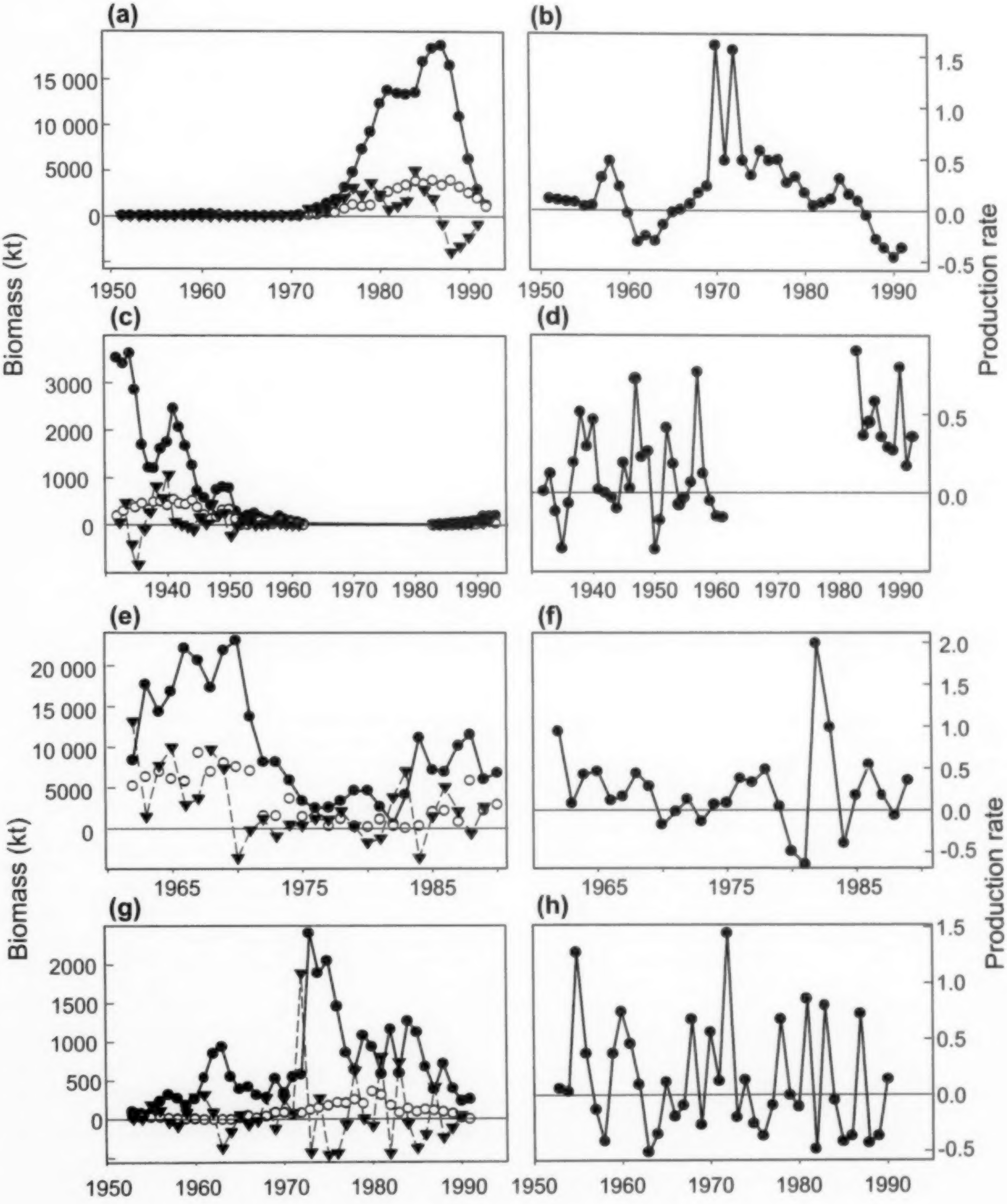


Fig. 6. Probability distributions of annual ISPR for (*a*) *E. mordax* (California), (*b*) *E. ringens* (Peru), (*c*) *S. melanostictus* (Japan), and (*d*) *S. sagax* (California). Bar plots are histograms rescaled so that frequencies sum to 1. Smooth lines are nonparametric density estimates (Venables and Ripley 1999) based on the same data.

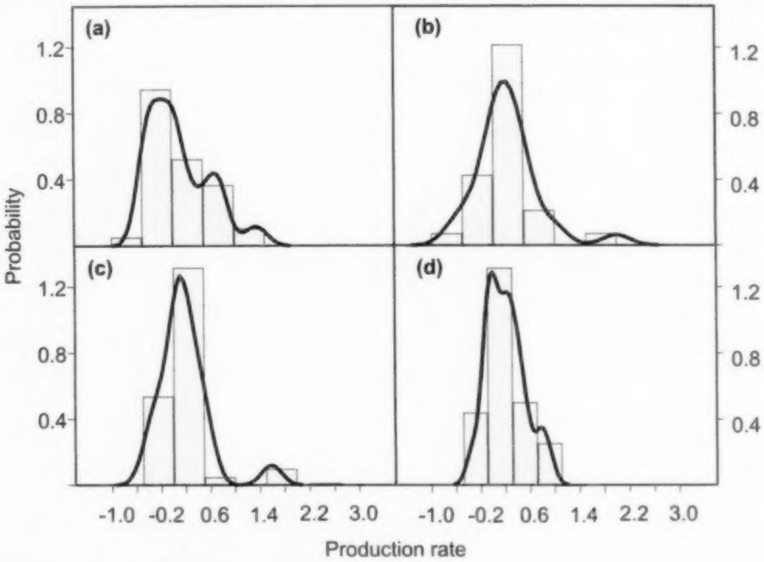


Table 4. Schaefer surplus production models $P_t = \alpha B + \beta B_t^2$ fit to biomass (B_t) and ASP (P_t) estimates by simple linear regression forced through the origin.

Stock and stock area	Residual df	α	P for α	β	P for β	Multiple R^2 (%)
Sardinops melanostictus (Japan)	39	0.29	0.041	-1.5×10^{-5}	0.10	16
S. sagax (California)	38	0.28	0.011	-9.2×10^{-5}	0.014	16
Engraulis ringens (Peru)	26	0.46	0.046	-1.4×10^{-5}	0.25	32
E. mordax (California)	36	0.31	0.11	-2.3×10^{-4}	0.052	11

Note: P values test the hypothesis that a parameter was zero.

lyzed the time series of catch data, ASP, ISPR, and biomass estimates for these stocks in detail.

Results for stocks with long time series show that declines in biomass tend to be pronounced whenever catch exceeds ASP for 5 or more years (Fig. 5). Strong biomass increases and highly productive regimes appear to be preceded by abrupt increases in ISPR. Statistical distributions for ISPR were skewed with relatively long tails over positive values and with modes around zero (Fig. 6).

Periods with positive ASP and ISPR were longer than periods with negative values (Fig. 5). Consistent with the autocorrelation results (see above), long-term time series plots (Fig. 5) of ISPR for sardine stocks (*S. melanostictus* off Japan and *S. sagax* off California) were smoother and less variable than for anchovy stocks (*E. ringens* off Peru and *E. mordax* off California). The longest "runs" of positive ASP and ISPR were 21 years for *S. melanostictus* off Japan, 10 years for *S. sagax* off California, 8 years for *E. ringens* off Peru, and 4 years for *E. mordax* off California (Fig. 5). The longest runs of negative ASP and ISPR were 4, 3, 2, and 3 years (Fig. 5). ASP and ISPR were positive in all years for five other sardine stocks, but the time series were shorter (≤22 years) (Tables 1 and 2). Duration of runs is likely to be underestimated in some cases because time series began or ended in the middle of a run.

Density-dependent relationships between ASP and biomass were explored by fitting crude Schaefer (1957) surplus production models $P_t = \alpha B_t + \beta B_t^2$ to annual production and biomass estimates by linear regression forced through the origin (Table 4; Fig. 7). Residuals from regression models for sardines were autocorrelated, with runs of years with ASP higher or lower than predicted by the model (Fig. 7).

Production per unit spawning area

ASP per unit spawning area was higher for sardines than for anchovies. Median ASP per unit of spawning area was 0.66 t·km^{-2}·year^{1} for anchovies (all years and all stocks) and 5.3 t·km^{-2}·year^{-1} for sardines (Table 5). Median ASP per unit spawning area for individual stocks ranged from –2.3 to 8.7 t·km^{-2}·year^{-1} for anchovies and from 1.4 to 16 t·km^{-2}·year^{-1} for sardines. Median ASP values per unit spawning area for *E. ringens* and *S. sagax* off northern Chile and southern Peru were relatively high but calculated using spawning area estimates based on general knowledge about spatial distribution during the spawning season rather than annual ichthyoplankton or acoustic surveys (Table 5).

Fig. 7. ASP and stock biomass for (*a*) *E. mordax* (California), (*b*) *E. ringens* (Peru), (*c*) *S. melanostictus* (Japan), and (*d*) *S. sagax* (California). Solid lines are Schaefer surplus production models fit by quadratic linear regression forced through the origin. Broken lines connect ASP and biomass estimates for consecutive years and the earliest year is identified in each plot. Data for *S. sagax* off California are missing for the years 1962–1982. Some points are obscured due to overlap.

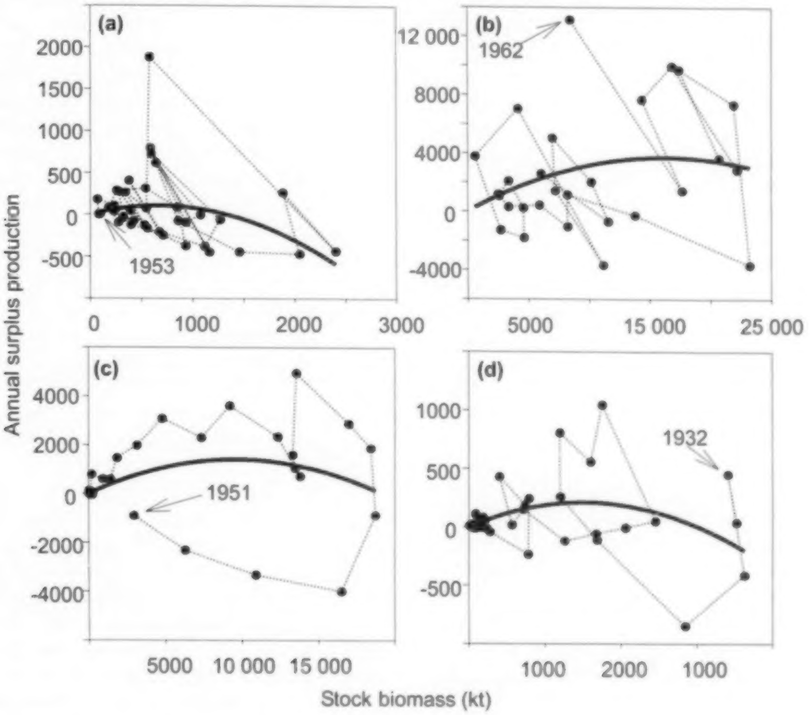


Table 5. ASP per unit spawning area (1000 $\text{kt}\cdot\text{year}^{1}\cdot\text{km}^{-2}$) for anchovy and sardine stocks.

Stock and stock area	n	$Q_{10\%}$	Median	$Q_{90\%}$	Type of spawning area estimate
Anchovies					
Engraulis anchoita (N. Patagonia)[a]	2	–3.4	–1.8	–0.060	AIS
E. anchoita (Uruguay – Buenos Aires)[a]	2	0.62	4.9	9.2	AIS
E. capensis (South Africa)[b]	10	–16.0	–2.3	24.0	AAS, AIS
E. encrasicolus (Bay of Biscay)[c]	6	–0.28	0.66	3.8	AIS
E. mordax (California)[d]	5	–3.0	–0.030	11.0	AIS
E. ringens (N. Chile – S. Peru)[c]	7	2.1	8.7	35.0	AD
All anchovies	32	–4.7	0.66	20.0	
Sardines					
Sardinops melanostictus (Japan)[c]	14	–11.0	6.0	18.0	AIS
S. sagax (South Africa)[b]	6	0.76	3.6	9.1	AAS, AIS
S. sagax (N. Chile – S. Peru)[c]	15	5.0	11.0	21.0	AD
S. sagax (California)[e]	7	1.6	2.0	4.0	AIS
Sardina pilchardus (Iberian Peninsula)[c]	2	15.0	16.0	16.0	AIS
Sardinella brasiliensis (Brazil)[c]	5	–1.3	1.4	5.3	AIS
All sardines	49	–1.9	5.3	17.0	

Note: Spawning areas were estimated either by annual ichthyoplankton surveys (AIS) or annual acoustic surveys (AAS) or from average distributions during the spawning season (AD).

[a] Sánchez et al. (1996).

[b] Barange et al. (1999).

[c] Unpublished data (Chile and Peru data from R. Serra, IFOP, Chile; Bay of Biscay and Iberian Peninsula data from A. Uriarte, AZTI, Spain, and C. Porteiro, Instituto Español de Oceanographia, Spain; Japan data from T. Wada, NRIFS, Japan; Brazil data from Y. Matsuura, Universidade de Sao Paulo, Brazil).

[d] Fiedler et al. (1986).

[e] Barnes et al. (1997).

Table 6. ASP at age over the life span of a hypothetical cohort of *S. sagax* off California based on data from Butler et al. (1993).

Age (years)	Annual natural mortality rate (year^{-1})	Individual body weight (g)	Cohort number	Cohort weight (g)	ASP (g·year^{-1})
0	13.4	0	1.000	0.0000	0.0810
1	0.4	53	0.00154	0.0810	0.0148
2	0.4	93	0.00103	0.0958	–0.0109
3	0.4	123	0.00069	0.0850	–0.0146
4	0.4	152	0.00046	0.0704	–0.0176
5	0.4	170	0.00031	0.0528	–0.0126
6	0.4	193	0.00021	0.0402	–0.0120
7	0.4	202	0.00014	0.0282	–0.0090
8	0.4	205	0.00009	0.0192	–0.0063
9	0.4	205	0.00006	0.0128	–0.0042
10	0.4	205	0.00004	0.0086	–0.0028
11	0.4	205	0.00003	0.0058	–0.0019
12	0.4	205	0.00002	0.0039	–0.0013
13	0.4	205	0.00001	0.0026	

Discussion

A total of eight anchovy and nine sardine stocks were studied, including populations from the southeast, southwest, and northeast Atlantic Ocean and the southeast, northeast, and northwest Pacific Ocean. Results provide a comprehensive overview of the interannual and interdecadal variability in ASP and ISPR for anchovies and sardines around the world.

Medians of annual ASP/B ratios were 0.15 for anchovies and 0.20 for sardines. As described above, $\bar{\bar{\delta}}$ values were 0.67 for anchovies and 0.85 for sardines. These results suggest that median ASP harvest rates of about 10% for anchovies and 17% for sardines would give increased and decreased stock biomass levels in the next year with equal probability. However, these calculations ignore differences among individual stocks. The relative benefits of median ASP harvest rate policies are unknown and should be a topic for future research through simulation.

Median average adjustment factors $\bar{\bar{\delta}}$ for catch in ASP calculations (Table 1) were 0.67 for anchovies and 0.85 for sardines. This suggests that 1 t of foregone catch under prevailing conditions in anchovy and sardine fisheries would result in 0.67 t of additional anchovy biomass or 0.85 t of additional sardine biomass after 1 year. Increases in biomass due to foregone catch would be in addition to gains from recruitment.

In the absence of better information, estimates of ASP per unit spawning area for anchovy and sardine stocks may be useful in setting management quotas based on annual estimates of spawning area or in estimating the potential yield of unfished sardine and anchovy stocks based on estimates of spawning area. However, it is important to remember that the spawning area and ASP estimates used in our analysis were 1 year out of phase because spawning in year t results in recruitment of age-1 fish at the beginning of season $t + 2$ that was recorded as production in season $t + 1$. Also, spawning area estimates were usually for periods near the peak spawning season that did not coincide in any fixed way to annual seasons used for productivity calculations. For *S. sagax* off California, for example, spawning area data for February–March 1994 were used to estimate productivity per unit of spawning area during the 1993 season, which extended from the beginning of July 1993 to the end of June 1994.

Higher variability in anchovies may be due to shorter life span and higher natural mortality rates that reduce the number of age-classes in anchovy relative to sardine stocks (e.g., Barange et al. 1999), differences in geographic distribution, or other differences in life history. Higher variability in anchovies has important practical and scientific ramifications. Based on available data, fishery managers should recognize that years with negative ASP occur in both anchovies and sardines but more commonly in anchovies. In addition, managers should expect periods with positive ASP to last longer in sardines than in anchovies. Scientists should recognize that differences in life history may make sardine and anchovy stocks in the same general area respond differently to the same climatic signal. For example, trends in ISPR for *E. mordax* and *S. sagax* off California are not similar. In general, ISPR rates are less variable for sardines than for anchovies, and sardines appear better able to buffer short-term environmental variability.

Instantaneous production rates for stocks with long time series were skewed to the right with a few relatively large values. Skewed distributions for ISPR with more positive values than negative values would be expected in exploited stocks due to density dependence. In addition, distributions of ISPR should resemble distributions for recruitment, which are usually skewed to the right (Hilborn and Walters 1992). In the worst-case scenario of no recruitment, distributions of ISPR are bounded below by the sum of instantaneous rates for (negative) natural mortality and (positive) somatic growth (i.e., in an unfished stock, $\rho \geq -M + G$ because $\rho = -M + G + R$ and the recruitment rate $R \geq 0$). The hard lower bound at $-M + G$ and the absence of a hard upper bound likely contribute to skewed distributions in ISPR.

ISPR may be better for identifying the onset of unfavorable and, particularly, favorable regimes (Luch-Belda et al. 1989, 1992; and defined above) than many other measures. Estimates of stock biomass are slower in reflecting regime shifts to unfavorable conditions because stock biomass includes age-classes that may persist for several years. Similarly, stock biomass may take time to build after the onset of favorable conditions. ASP estimates respond slowly to favorable regime shifts when biomass is low because even high ISPR gives little ASP under low biomass conditions. Catch data are the least sensitive indicator of regime shifts because economic factors, policy, and fishery management procedures affect catches. For example, catches may remain high after regime shifts from favorable to unfavorable conditions, even after biomass falls, because of market demand or lack of fishery management or because policies for setting total allowable catch limits aim to maintain stable catches from year to year.

ISPR values are more sensitive than other measures, but sensitivity comes at the cost of more variability. ISPR in any particular year during an unfavorable regime may be as high as typical values during favorable periods, and vice versa. For example, compare ISPR for *S. melanostictus* off Japan during 1958 when conditions were generally not favorable with ISPR during 1974–1980 when conditions were generally favorable. ISPR is more sensitive and better for identifying the timing of regime shifts retrospectively, but several years of high or low ISPR might be required to determine whether a regime shift has just taken place.

For example, a shift to favorable regime conditions is evident in ISPR for *S. melanostictus* off Japan by 1970 but not reflected in biomass and ASP estimates until at least 1972 and not evident in catch data until at least 1976. A shift to unfavorable conditions in the same stock was evident in the ISPR and ASP by 1986 but not evident in biomass estimates until at least 1990 and never clearly evident in the catch data. For *S. sagax* off California, a favorable regime during the 1980s and 1990s is clearly evident in the ISPR estimates but not evident in biomass, ASP estimates, or catch data. For *E. ringens* off Peru, a period of unfavorable conditions is evident in ISPR and ASP estimates for 1970, while catches and biomass were at maximum levels. ISPR increased to near normal levels by 1975, long before stock biomass, catches, and ASP showed substantial increases. Finally, the period of high biomass and apparently productive conditions for *E. mordax* off California during the 1973–1977 was due to a single year of high production and production rates in 1972 followed by several years of mostly negative production and production rates.

As described above, our analysis focused on environmental effects on ASP and ISPR, and we did not partition variability into density-dependent and density-independent (i.e., environmental) components. However, preliminary production model results in this paper indicate that environmental effects likely contribute more variance to ASP and ISPR than density-dependent effects for anchovy and sardine stocks in our study. Production models fit to long time series of production estimates for *S. melanostictus* off Japan, *S. sagax* off California, *E. ringens* off Peru, and *E. mordax* off California explained relatively low to moderate proportions (average 19%) of the total variance in production. Parameter estimates for the production models were usually statistically significant but were misleading in at least two cases. The production models for Japanese and California sardine imply that B_{MSY} is in the vicinity of 1×10^7 and 1×10^6 t, respectively. In contrast, Wada and Jacobson (1998) determined that carrying capacity (and B_{MSY}) changed by a factor of about 75 following regime shifts in Japanese sardine. Similarly, Jacobson and MacCall (1995) found that B_{MSY} for California sardine changed from about 270 000 to 1800 t when 3-year average temperatures changed by 0.8°C.

Based on recruitment success in Japanese sardine and California sardine, Wada and Jacobson (1998) hypothesized that density-dependent effects on recruitment (an important component of production) "typically exist for *Sardinops* spp. but can only be measured using long time series of data with large (thousand fold) changes in abundance." By analogy with recruitment, we hypothesize that density-dependent effects on ASP and ISPR are best measured, even in long time series, using relatively complex models that accommodate environmental variability.

There are difficulties in using fisheries data to track environmental changes that are difficult to circumvent and not to be ignored. Most time series of production estimates are shorter than required (about 30 years) to completely appreciate variability. Catch data are often available for longer periods of time but, as shown above, are relatively insensitive to climate change.

Another disadvantage is that fishery data provide little information about ages younger than taken in the fishery (e.g., eggs, larvae, and juveniles). A quick calculation (Table 6) based on "best" estimates of natural mortality rates and stage durations for age-0 fish and weight at age data (all from tables 2 and 3 in Butler et al. 1993) with $M = 0.4\cdot\mathrm{year}^{1}$ for ages 1+ (assumed in stock assessment work) shows that ASP is negative for a an unexploited cohort of *S. sagax* off California after age 2 and that the bulk of positive production occurs at age 0 (as individuals grow from eggs to stage-III juveniles at age 1). Thus, ASP in our study due to a strong or weak recruitment of age-1 fish might have resulted from environmental factors during the previous year (while the cohort was age 0) or 2 years previous (during gametogenesis). The same calculation shows that the effect of a large persistent year-class on ASP is negative after age 2.

Acknowledgements

This research is the work of Working Group 3 (Comparative Population Dynamics) of the Small Pelagic Fish and Climate Change (SPACC) Program of the Global Ocean Ecosystem Dynamics (GLOBEC) Program (www.globec.org). It was supported by GLOBEC, the Scientific Committee on Ocean Research (SCOR), the U.S. National Science Foundation, the Inter-America Institute for Global Change Research (IAI), and the agencies and universities that employ the authors. The School for Marine Science and Technology at the University of Massachusetts provided a venue and support for a working group meeting. We thank Kevern Cochrane and an anonymous reviewer for comments that substantially improved the manuscript.

References

Barange, M., Hampton, I., and Roel, B.A. 1999. Trends in the abundance and distribution of anchovy and sardine on the South

African continental shelf in the 1990s, deduced from acoustic surveys. S. Afr. J. Mar. Sci. **21**: 367–392.

Barnes, J.T., Jacobson, L.D., MacCall, A.D., and Wolf, P. 1992. Recent population trends and abundance estimates for the Pacific sardine (*Sardinops sagax*). Calif. Coop. Oceanic Fish. Invest. Rep. **33**: 60–75.

Barnes, J.T., Yaremko, M., Jacobson, L.D., Lo, N.C.H., and Stehly, J. 1997. Status of the Pacific sardine (*Sardinops sagax*) resource in 1996. NOAA Tech. Memo. NMFS, **237**: 1–17.

Blaxter, J.H.S., and Hunter, J.H. 1982. The biology of clupeoid fishes. Adv. Mar. Biol. **20**: 1–223.

Butler, J.L., Smith, P.E., and Lo, N.C.H. 1993. The effect of natural variability of life-history parameters on anchovy and sardine population growth. Calif. Coop. Oceanic Fish. Invest. Rep. **34**: 104–111.

Butterworth, D.S., De Oliveira, J.A.A., and Cochrane, K.L. 1993. Current initiatives in refining the management procedure for the South African anchovy resource. *In* Proceedings of the International Symposium on Management Strategies for Exploited Fish Populations. *Edited by* G. Kruse, D.M. Eggers, R.J. Marasco, C. Pautzke, and T.J. Quinn II. Alaska Sea Grant College Program Rep. No. 93-02. University of Alaska Fairbanks, Fairbanks, Alaska. pp. 439–474.

Cergole, M.C. 1995. Stock assessment of Brazilian sardine, *Sardinella brasiliensis*, of the south-eastern coast of Brazil. Sci. Mar. **59**: 597–610.

Cisneros-Mata, M.A., Nevárez-Martínez, M.O., and Hammann, M.G. 1995. The rise and fall of the Pacific sardine, *Sardinops sagax caeruleus* Girard, in the Gulf of California, Mexico. Calif. Coop. Oceanic Fish. Invest. Rep. **36**: 136–143.

Cury, P., Bakun, A., Crawford, R.J.M., Jarre, A., Quiñones, R.A., Shannon, L.J., and Verheye, H.M. 2000. Small pelagics in upwelling systems: patterns of interaction and structural changes in "wasp-waist" ecosystems. ICES J. Mar. Sci. **57**: 603–618.

Deriso, R.B., Barnes, J.T., Jacobson, L.D., and Arenas, P.R. 1996. Catch-at-age analysis for Pacific sardine (*Sardinops sagax*). Calif. Coop. Oceanic Fish. Invest. Rep. **37**: 175–187.

Felix-Uraga, R., Alvarado-Castillo, R.M., and Carmona-Piña, R. 1996. The sardine fishery along the western coast of Baja California, 1981–1994. Calif. Coop. Oceanic Fish. Invest. Rep. **37**: 188–192.

Fiedler, P.C., Methot, R.D., and Hewitt, R.P. 1986. Effects of California El Niño 1982–1984 on the northern anchovy. J. Mar. Res. **44**:317–338.

Hayashi, S. 1961. Fishery biology of the Japanese anchovy, *Engraulis japonica*. Bull. Tokai Reg. Fish. Res. Lab. **31**: 145–268.

Hilborn, R., and Walters, C.J. 1992. Quantitative fisheries stock assessment: choice, dynamics and uncertainty. Routledge, Chapman and Hall, Inc., New York.

Hunter, J.R., and Alheit, J. (*Editors*). 1995. International GLOBEC Small Pelagic Fishes and Climate Change Program. Report of the First Planning Meeting, La Paz, Mexico, June 20–24, 1994. GLOBEC Rep. No. 8.

International Council for the Exploration of the Sea. 1996. Report of the working group on the assessment of mackerel, horse mackerel, sardine and anchovy. ICES CM1997/Assess:3. pp. 279–320.

Jacobson, L.D., and MacCall, A.D. 1995. Stock–recruitment models for Pacific sardine (*Sardinops sagax*). Can. J. Fish. Aquat. Sci. **52**: 566–577.

Jacobson, L.D., Lo, N.C.H., and Barnes, J.T. 1994. A biomass-based assessment model for northern anchovy, *Engraulis mordax*. Fish. Bull. U.S. **92**: 711–724.

Jacobson, L.D., Lo, N.C.H., Herrick, S.F., Jr., and Bishop, T. 1995. Spawning biomass of the northern anchovy in 1995 and status of the coastal pelagic fishery during 1994. Southwest Fish. Sci. Cent. Admin. Rep. LJ-95-11.

Lluch-Belda, D., Crawford, R.J.M., Kawasaki, T., MacCall, A.D., Parrish, R.H., Schwartzlose, R.A., and Smith, P.E. 1989. World-wide fluctuations of sardine and anchovy stocks: the regime problem. S. Afr. J. Mar. Sci. **8**: 195–205.

Lluch-Belda, D., Schwartzlose, R.A., Serra, R., Parrish, R.H., Kawasaki, T., Hedgecock, D., and Crawford, R.J.M. 1992. Sardine and anchovy regime fluctuations of abundance in four regions of the worlds oceans: a workshop report. Fish. Oceanogr. **1**: 339–347.

MacCall, A.D. 1978. A note on production modeling of populations with discontinuous reproduction. Calif. Fish Game, **64**: 225–227.

Methot, R.D. 1989. Synthetic estimates of historical abundance and mortality for northern anchovy. Am. Fish. Soc. Symp. **6**: 66–82.

Opsomer, J.D., and Conrad, J.M. 1994. An open-access analysis of the northern anchovy fishery. J. Environ. Econ. Manage. **27**: 21–37.

Pope, J.G. 1972. An investigation of the accuracy of virtual population analysis using cohort analysis. Int. Comm. Northwest Atl. Fish. Res. Bull. **9**: 65–74.

Ricker, W.E. 1975. Computation and interpretation of biological statistics of fish populations. Bull. Fish. Res. Board Can. No. 191.

Sánchez, R.P., Pájaro, M., and Maachi, G.G. 1996. The application of the daily egg production method to the assessment of SW Atlantic anchovy (*Engraulis achoita*), spawning biomass off Argentina. ICES CM 1996/H:29.

Schaefer, M.B. 1957. A study of the dynamics of the fishery for yellowfin tuna in the eastern tropical Pacific Ocean. Inter-Am. Trop. Tuna Comm. Bull. **2**: 245–285.

Schwartzlose, R.A., Alheit, J., Bakun, A., Baumgartner, T.R., Cloete, R., Crawford, R.J.M., Fletcher, W.J., Green-Ruiz, Y., Hagen, E., Kawasaki, T., Lluch-Belda, D., Lluch-Cota, S.E., MacCall, A.D., Matsuura, Y., Nevarez-Martinez, M.O., Parrish, R.H., Roy, C., Serra, R., Shust, K.V., Ward, M.N., and Zuzunaga, J.A. 1999. Worldwide large-scale fluctuations of sardine and anchovy populations. S. Afr. J. Mar. Sci. **21**: 289–347.

Sissenwine, M.P., and Shepherd, J.G. 1987. An alternate perspective on recruitment overfishing and biological reference points. Can. J. Fish. Aquat. Sci. **44**: 913–918.

Trenberth, K.E. 1990. Recent observed interdecadal climate changes in the Northern Hemisphere. Bull. Am. Meteorol. Soc. **71**: 988–993.

Venables, W.N., and Ripley, B.D. 1999. Modern applied statistics with S-PLUS. Springer-Verlag, New York.

Wada, T., and Jacobson, L.D. 1998. Regimes and stock–recruitment relationships in Japanese sardine (*Sardinops melanostictus*), 1951–1995. Can. J. Fish. Aquat. Sci. **55**: 2455–2463.

Appendix A

We want to measure annual surplus production (ASP) for ages 1+ using eq. 3, but official catch records may include catch of fish age 0 so that $C_t = C_{t,0} + C_{t,1+}$. With catch of age 0 fish, the calculation in eq. 3 becomes

$$\text{(A1)}\quad P_t^* = B_{t+1} - B_t + \bar{\bar{\delta}}(C_{t,0} + C_{t,1+}) = P_t + \bar{\bar{\delta}} C_{t,0}$$

and the relative error in calculation of ASP due to catch of age-0 fish is

$$\text{(A2)}\quad \frac{(P_t^* - P_t)}{P_t} = \frac{\bar{\bar{\delta}} C_{t,0}}{P_t}.$$

Appendix B

The instantaneous annual surplus production rate (ISPR) is the sum of rates for recruitment (R_t), somatic growth (G_t), and natural mortality (M). Following MacCall (1978):

$$\text{(B1)} \quad B_{t+1} = B_t \mathrm{e}^{R_t + G_t - M - F_t}$$

where F_t is the fishing mortality rate. MacCall (1978) assumed that recruitment was a seasonal "pulse." We assume that recruitment is a continuous process (because most sardines and anchovies recruit throughout the year), but the difference in convention is not important. With no fishing during year t:

$$\text{(B2)} \quad B^*_{t+1} = B_t \mathrm{e}^{R_t + G_t - M} \ .$$

As shown above, $B^*_{t+1} = B_t \mathrm{e}^{\rho_t}$, where ρ_t is the ISPR. Substituting terms for B^*_{t+1} gives $\rho_t = R_t + G_t - M$ and $B_{t+1} = B^*_t \, \mathrm{e}^{-F_t}$.

The relationship between ISPR, F_t, and catch is given by Baranov's catch equation (Ricker 1975):

$$\text{(B3)} \quad C_t = \frac{F_t}{Z_t}(1 - \mathrm{e}^{-Z_t})B_t$$

where $Z_t = F_t - \rho_t$. The adjustment factor $\bar{\bar{\delta}}$ converts units of catch to units of ASP (see above) so that $B^*_{t+1} - B_t = \bar{\bar{\delta}} C$.

Appendix B references

MacCall, A.D. 1978. A note on production modeling of populations with discontinuous reproduction. Calif. Fish Game, **64**: 225–227.

Ricker, W.E. 1975. Computation and interpretation of biological statistics of fish populations. Bull. Fish. Res. Board Can. No. 191.

National Research Council Canada
Conseil national de recherches Canada

Copyright Transfer

ASSIGNMENT OF COPYRIGHT

Ms. No. ____________

To: NATIONAL RESEARCH COUNCIL OF CANADA ("NRC") Research Press

From: ______________________________
____
____
____
(name and address of author signing this form)

This refers to the manuscript entitled: ______________________________
____

written by myself and the following additional author(s): ______________________________
____

for publication in: ______________________________

1. I declare that the list of authors fairly conforms to academic conventions about who should be listed. I declare that the manuscript, or its equivalent, has not been submitted for publication elsewhere. If it is accepted for publication by the National Research Council of Canada, I will ensure that it, or its equivalent, is not submitted for publication elsewhere.

2. I declare that I am (check ONE):

☐ (a)	the sole owner of the copyright relating to the manuscript,	and →	I **assign** to NRC the entire copyright.
☐ (b)	a part owner of the copyright, in conjunction with interests held by co-authors or their employers,	and →	I **assign** to NRC my entire interest in the copyright, and I expect the co-authors to also sign a form like this one.
☐ (c)	an agent of my employer with authority to assign the copyright owned by the employer, who is: ______________________,	and →	I **assign** to NRC, on behalf of my employer, my employer's entire interest in the copyright relating to the manuscript, and I expect the co-authors to also sign a form like this one.
☐ (d)	a public servant in Canada (federal, provincial or territorial) or the United Kingdom, so that the copyright is owned by Her Majesty,	and →	I have obtained the approval of my employer, according to the terms of my employment, to grant NRC **non-exclusive permission** to publish the manuscript and administer the copyright.
☐ (e)	a public servant of the United States of America, and subject to the law that creates no U.S. copyright for the manuscript,	and →	I **assign** to NRC, on behalf of my employer, my employer's entire interest in any copyright that may exist outside the U.S., and I expect the co-authors to sign a form like this one.

3. I understand that my submission of this document does not guarantee publication, and that publication will not occur until **all authors** have provided a copyright transfer. If the manuscript is not published within 2 years after I sign this, the assignment is revoked.

Signed at ______________________________ on ______________________
(City and Province/State) (Date)

____
(Signature)

[*Standard NRC form; alterations not acceptable.*]

Comments on Copyright Transfer
(with extracts from the *Copyright Act*, Revised Statutes of Canada 1985, chapter C-42, as amended)

The publisher is not permitted to publish the manuscript without permission or an assignment of ownership of copyright. For greatest flexibility, and to be prepared to deal with future uses of the published material, the publisher requires ownership.

The owner may assign the right, but that assignment must be in writing:

13.(4) *The owner of the copyright in any work may assign the right, either wholly or partially, and either generally or subject to territorial limitations, and either for the whole term of the copyright or for any other part thereof, and may grant any interest in the right by licence, but no assignment or grant is valid unless it is in writing signed by the owner of the right in respect of which the assignment or grant is made, or by his duly authorized agent.*

The owner may be the author, but in many cases, the owner is the author's employer:

13.(3) *Where the author of a work was in the employment of some other person under a contract of service or apprenticeship and the work was made in the course of his employment by that person, the person by whom the author was employed shall, in the absence of any agreement to the contrary, be the first owner of the copyright, but where the work is an article or other contribution to a newspaper, magazine or similar periodical, there shall, in the absence of any agreement to the contrary, be deemed to be reserved to the author a right to restrain the publication of the work, otherwise than as part of a newspaper, magazine or similar periodical.*

The only exception to taking ownership arises when the manuscript is covered by Crown copyright owned by a Canadian government.

12. *Without prejudice to any rights or privileges of the Crown, where any work is, or has been, prepared or published by or under the direction or control of Her Majesty or any government department, the copyright in the work shall, subject to any agreement with the author, belong to Her Majesty and in that case shall continue for the remainder of the calendar year of the first publication of the work and for a period of fifty years following the end of that calendar year.*

Governments, and in particular the federal government, do not have a simple mechanism for assigning ownership of this particular type of Crown asset. Therefore, when an author is a public servant so that there is Crown copyright in the manuscript, a simple permission to use is acceptable to the publisher, if it is granted by a person with appropriate authority.

Conseil national de recherches
Canada

National Research Council
Canada

Cession du droit d'auteur

CESSION DU DROIT D'AUTEUR

Document n° ________

Au : CONSEIL NATIONAL DE RECHERCHES DU CANADA (« CNRC ») Presses scientifiques

De : ______________________________

____

____

____

(nom et adresse de l'auteur qui signe le présent formulaire)

La présente cession porte sur le document intitulé : ______________________________

____

rédigé par moi, en collaboration avec l'auteur/les auteurs suivant(s) : ______________________________

____

aux fins de publication dans : ______________________________

1. Je déclare que la liste des auteurs ci-dessus respecte les pratiques usuelles de rédaction savante quant aux personnes dont le nom doit être cité. Je déclare que le document ou son équivalent n'a pas été soumis à quelque autre organisme aux fins de publication. Si le CNRC accepte de publier le document, je m'assurerai que celui-ci ou son équivalent n'est soumis à aucun autre organisme aux fins de publication sans en obtenir la permission préalable du CNRC.

2. Je déclare être (UN SEUL choix) :

☐	(a)	le seul titulaire de droit d'auteur afférent au document,	et →	je **cède** le droit d'auteur intégral au CNRC.
☐	(b)	un titulaire partite du droit d'auteur, de concert avec des coauteurs ou leurs employeurs,	et →	je **cède** ma part du droit d'auteur au CNRC et m'attends à ce que les coauteurs signent une entente comparable.
☐	(c)	un agent de mon employeur ayant l'autorité de céder le droit d'auteur appartenant à l'employeur, nommément : ______________________,	et →	je **cède** au CNRC, au nom de mon employeur, la part entière du droit d'auteur afférent au document appartenant à mon employeur, et m'attends à ce que les coauteurs signent une entente comparable.
☐	(d)	un fonctionnaire du Canada (fédéral, provincial ou territorial) ou du Royaume-Uni, donc le droit d'auteur appartient à Sa Majesté,	et →	j'ai obtenu de mon employeur, en vertu de mes conditions d'emploi, l'autorisation d'accorder au CNRC la **permission non exclusive** de publier le document et de gérer le droit d'auteur.
☐	(e)	un fonctionnaire des États-Unis d'Amérique, donc sujet à la loi qui n'associe aucun droit d'auteur américain au document,	et →	je **cède** au CNRC, au nom de mon employeur, la part entière de quelque droit d'auteur en vigueur ailleurs qu'aux É.-U. appartenant à mon employeur, et m'attends à ce que les coauteurs signent une entente comparable.

3. Je comprends que la soumission du document ne garantit aucunement sa publication et que la publication ne pourra être réalisée tant que **tous les auteurs** n'auront pas signé une cession du droit d'auteur. Si le document n'est pas publié dans les deux années suivant ma signature des présentes, la cession sera automatiquement révoquée.

Signé à ______________________________, le ______________________

(ville et province/état) (date)

____

(signature)

[*Formulaire normalisé du CNRC; aucune modification permise.*]

Commentaires sur la cession du droit d'auteur
(accompagnés d'extraits de la *Loi sur le droit d'auteur*, Lois révisées du Canada 1985, chapitre C-42 modifié)

L'éditeur ne peut publier un document sans la permission ou une cession du droit d'auteur. Pour des raisons de souplesse et en vue de l'utilisation future du document publié, l'éditeur doit devenir titulaire du droit d'auteur.

Le titulaire peut céder le droit, mais cette cession doit être faite par écrit :

13.(4) *Le titulaire du droit d'auteur sur une oeuvre peut céder ce droit, en totalité ou en partie, d'une façon générale, ou avec des restrictions territoriales, pour la durée complète ou partielle de la protection; il peut également concéder, par une licence, un intérêt quelconque dans ce droit; mais la cession ou la concession n'est valable que si elle est rédigée par écrit et signée par le titulaire du droit qui en fait l'objet, ou par son agent dûment autorisé.*

Le titulaire peut être l'auteur, mais dans plusieurs cas, le titulaire est l'employeur de l'auteur :

13.(3) *Lorsque l'auteur est employé par une autre personne en vertu d'un contrat de louage de service ou d'apprentissage, et que l'oeuvre est exécutée dans l'exercice de cet emploi, l'employeur est, à moins de stipulation contraire, le premier titulaire du droit d'auteur; mais lorsque l'oeuvre est un article ou un autre contribution, à un journal, à une revue ou à un périodique du même genre, l'auteur, en l'absence de convention contraire, est réputé posséder le droit d'interdire la publication de cette oeuvre ailleurs que dans un journal, une revue ou un périodique semblable.*

La seule exception dans la cession du droit d'auteur est lorsque le document est associé au droit d'auteur de la Couronne, qui appartient au gouvernement du Canada.

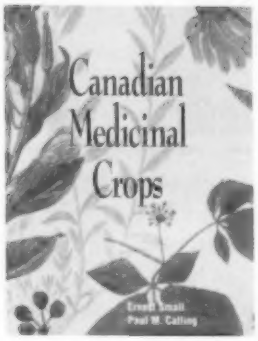
12. *Sous réserve de tous les droits ou privilèges de la Couronne, le droit d'auteur sur les oeuvres préparées ou publiées par l'entremise, sous la direction ou la surveillance de Sa Majesté ou d'un ministère du gouvernement, appartient, sauf stipulation conclue avec l'auteur, à Sa Majesté et, dans ce cas, il subsiste jusqu'à la fin de la cinquantième année suivant celle de la première publication de l'oeuvre.*

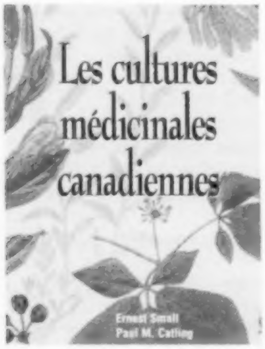
Les gouvernements, en particulier le gouvernement fédéral, ne disposent pas d'un mécanisme simple de cession de droit d'auteur pour ce genre de biens de la Couronne. C'est pourquoi lorsqu'un auteur est un fonctionnaire et que, par conséquent, le droit d'auteur appartient à la Couronne, une simple permission d'usage est suffisante pour l'éditeur, pour autant que celle-ci ait été octroyée par une personne jouissant de l'autorité nécessaire pour ce faire.

Canadian Medicinal Crops

By Ernest Small and Paul M. Catling

Softcover
ISBN 0-660-17534-7
NRC 42252
$29.95 CAN. Other countries $29.95 US
29,95 $CAN. À l'étranger 29,95 $US





Les cultures médicinales canadiennes

Par Ernest Small et Paul M. Catling

Édition brochée
ISBN 0-660-96380-9
NRC 42253
$29.95 CAN. Other countries $29.95 US
29,95 $CAN. À l'étranger 29,95 $US

Orders may be submitted by mail or fax to:
Les commandes peuvent être soumises par courrier ou par télécopieur à :

Order Office/Bureau des commandes
NRC Research Press/Presses scientifique du CNRC
National Research Council/Conseil national de recherches
Ottawa, ON K1A 0R6, Canada
Telephone/Téléphone : (613) 990-2254
Fax/Télécopieur :(613) 952-7656
E-mail/Courriel: research.journals@nrc.ca
http://researchpress.nrc.ca

PLEASE PRINT/LETTRES MOULÉES

☐ Payment enclosed Paiement inclus ☐ Charge to/ Imputer à ☐ Visa ☐ MasterCard

Account no./Nº de compte ☐☐☐☐☐☐☐☐☐☐☐☐☐☐☐☐ Expiry date/Date d'expiration ☐☐☐☐

Name/Nom

Signature

Address/Adresse

City/Ville Province

Postal code/Code postal Country/Pays

Telephone/Téléphone Fax/Télécopieur

E-mail/Courriel:

Prepayment required unless order is accompanied by a purchase order number. Cheques or money orders should be made payable to : "Receiver General for Canada, Credit NRC". For all orders outside Canada, rates are in U.S. Dollars. All sales are final.
Les commandes doivent être payées d'avance à moins qu'elles ne soient accompagnées d'un numéro de bon de commande. Les chèques ou mandats doivent être libellés au « Receveur général du Canada, crédit du CNRC ». Toutes les ventes sont finales.

Flora of the Yukon Territory

William J. Cody

643 pages
December/décembre 1996
Hardcover/Édition reliée
ISBN 0-660-16406-X
NRC/CNRC 40389
$79.95/79,95 $
Softcover/Édition brochée
ISBN 0-660-15898-1
NRC/CNRC 40390
$49.95/49,95 $

This book treats 1112 species representing 80 families of vascular plants that have been found in or near the Yukon Territory. Descriptions and keys provide information on families, genera, species, subspecies, and varieties. All species are illustrated and dot maps depict known distributions within the Yukon Territory. Visit our Web site at http://www.nrc.ca/cisti/journals/40390/flora.html to view a short chapter of *Flora of the Yukon Territory.*

Ce traité décrit 1112 espèces de plantes vasculaires, représentant 80 familles, qui se retrouvent au Yukon ou aux environs du Territoires. Les descriptions et les clefs présentées offrent de l'information sur les familles, les genres, les espèces, les sous-espèces et les variétés. Toutes les espèces sont illustrées et des cartes de points illustrent la répartition connue au Yukon. Visitez http://www.nrc.ca/cisti/journals/40390/flora_f.html pour afficher un court chapitre du livre.

To order copies of this publication/Pour commander des exemplaires de ce livre

- Telephone: (613) 993-0151
- Fax: (613) 952-7656
- Mail orders: Monograph Orders, NRC Research Press, National Research Council Canada, Ottawa, Ontario K1A 0R6, Canada
- Internet e-mail: research.journals@nrc.ca
- World Wide Web: http://www.nrc.ca/cisti/journals/formmono.html

Canadian customers please add 7% GST. Orders from Quebec add a further 6.5% QST to the total price including GST. Make cheque or money order payable to "Receiver General for Canada, credit NRC". Note that for all orders outside Canada, rates are in U.S. dollars. All sales are final.

- Téléphone : (613) 993-0151
- Télécopieur : (613) 952-7656
- Par la poste : Monographies, Presses scientifiques du CNRC, Conseil national de recherches Canada, Ottawa (Ontario) K1A 0R6, Canada
- Courrier électronique : research.journals@nrc.ca
- World Wide Web : http://www.nrc.ca/cisti/journals/formmofr.html

Les clients canadiens doivent ajouter la TPS de 7 %. Les clients du Québec doivent ajouter 6,5 % de TVQ au montant total, une fois la TPS additionnée. Libellez le chèque ou le mandat à l'ordre du «Receveur général du Canada, au crédit du CNRC». Pour les clients non canadiens, les prix sont en dollars américains. Aucun remboursement.

Please send me copies of/Veuillez me faire parvenir des exemplaires du *Flora of the Yukon Territory*
_____ (hardcover/édition reliée); _____ (softcover/édition brochée).

PLEASE PRINT/LETTRES MOULÉES
☐ Payment enclosed/Paiement inclus ☐ Charge to/Imputer à ☐ Visa ☐ MasterCard
Account number/Nº de compte ☐☐☐☐☐☐☐☐☐☐☐☐☐☐☐☐ Expiry date/Date d'expiration________
Name/Nom
Signature/Signature
Address/Adresse
City/Ville Province/Province
Postal code/Code postal Country/Pays
Telephone/Téléphone Fax/Télécopieur
e-mail/Courrier électronique Date of order/Date de la commande

NRC Research Press

Monograph Program

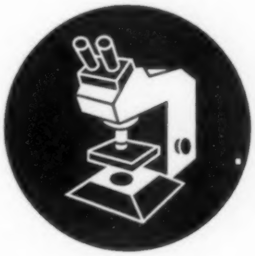
Presses scientifiques du CNRC

Programme de monographies

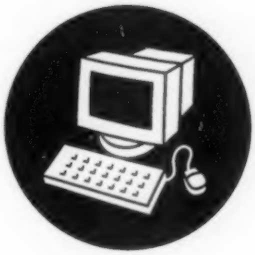
NRC·CNRC

At the Dawn of the Third Millennium, Which is the Best Way to Stay on Top of Advances in Limnology?

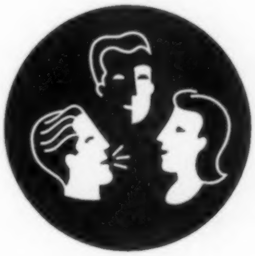
a)



b)



c)



Correct answer is c). As surprising as it may sound, in this age of state-of-the-art research facilities, infinite computing power and rapid communication, scientific presentations and face-to-face exchanges remain the best way to stay on top of the most recent advances in limnology. Intimate gatherings, such as the annual meeting of the Society of Canadian Limnologists (SCL), provide the ideal forum. The next SCL annual meeting is in Vancouver, British Columbia, January 3-5th 2002, together with the Canadian Conference for Fisheries Research.

All members of SCL are entitled to a 40% discount on subscription rates to the Canadian Journal of Fisheries and Aquatic Sciences. As a student, you are eligible for awards from the Clemens-Rigler travel fund to attend the annual meeting.

Details on the Society and our next conference can be found at **www.limnology.org/canada**

The Society of Canadian Limnologists
La Société Canadienne de Limnologie

ANNOUNCEMENT

The **Canadian Conference for Fisheries Research** will be held 3–5 January 2002 in Vancouver, British Columbia, Canada.

Where: The Empire Landmark Hotel and Conference Centre
1400 Robson Street, Vancouver, BC Y6G 1B9
Telephone: 800-830-6144

Themes: Climate Change Impacts on Aquatic Ecosystems (CCFFR/SCL)
Fish Biology: Behaviour, Conservation, and Evolutionary Genetic (CCFFR)
W. E. Ricker: Shaping a Science (CCFFR)
Aquatic Ecological Research: Scaling, Society, and Priorities (CCFFR/SCL)
Productive Capacity of Aquatic Habitat (CCFFR)

Call for papers: Abstracts in electronic form must be submitted to the program chair (Reistj@dfo-mpo.gc.ca) by 15 October 2001 (preferred) or 31 October 2001 (at the latest).

Funding: Graduate students may apply to the Clemens-Rigler Travel Fund.

Web site: For further information on the conference, registration details, program schedules, and updates, visit the CCFFR Web site at www.phys.ocean.dal.ca/ccffr/

Note: The deadline for the special rate at the hotel is 1 November 2001. Early submission of abstract/poster for acceptance will ensure adequate time for hotel booking.

ANNONCE

La **Conférence canadienne sur la recherche des pêches** aura lieu du 3 au 5 janvier 2002 à Vancouver, Colombie-Britannique, Canada.

Endroit : Hôtel et centre de conférence Empire Landmark
1400, rue Robson, Vancouver, BC Y6G 1B9
Téléphone : 800-830-6144

Thèmes : Répercussions des changements climatiques sur les écosystèmes aquatiques (CCRP/SCL)
Biologie du poisson : comportement, conservation et génétique évolutive (CCRP)
W. E. Ricker : façonner une science (CCRP)
Recherche sur l'écologie aquatique : barème, société et priorités (CCRP/SCL)
Capacité de production de l'habitat aquatique (CCRP)

Appel pour contributions : Les résumés en format électronique doivent êtres soumis à la chaise du programme (Reistj@dfo-mpo.gc.ca) par le 15 octobre 2001 (préféré) ou le 31 octobre 2001 (au dernier).

Financement : Les diplômés peuvent faire une demande de financement auprès de la Caisse de voyage Clemens-Rigler.

Site web : Pour obtenir de plus amples renseignements sur la conférence, l'inscription, l'horaire et les mises à jour, veuillez consulter le site Web de la CCRP à l'adresse http://www.phys.ocean.dal.ca/ccffr/

Nota : La date limite pour le taux spécial à l'hôtel est le 1 novembre 2001. Le soumission première d'abstrait/affiche pour l'acceptation assurera le temps suffisant pour la réservation d'hôtel.

Contents (concluded) / Table des matières (suite et fin)

Benjamin H. Letcher and Tim L. King 1812–1821 Parentage and grandparentage assignment with known and unknown matings: application to Connecticut River Atlantic salmon restoration

David B. Donald, Rolf D. Vinebrooke, R. Stewart Anderson, Jim Syrgiannis, and Mark D. Graham 1822–1830 Recovery of zooplankton assemblages in mountain lakes from the effects of introduced sport fish

James H. Petersen and James F. Kitchell 1831–1841 Climate regimes and water temperature changes in the Columbia River: bioenergetic implications for predators of juvenile salmon

D.J. McGlashan, J.M. Hughes, and S.E. Bunn 1842–1852 Within-drainage population genetic structure of the freshwater fish *Pseudomugil signifer* (Pseudomugilidae) in northern Australia

Michael M. Hansen, Einar E. Nielsen, Dorte Bekkevold, and Karen-Lise D. Mensberg 1853–1860 Admixture analysis and stocking impact assessment in brown trout (*Salmo trutta*), estimated with incomplete baseline data

Peter T. Stevick, Per J. Palsbøll, Tim D. Smith, Mark V. Bravington, and Philip S. Hammond 1861–1870 Errors in identification using natural markings: rates, sources, and effects on capture–recapture estimates of abundance

PERSPECTIVES PERSPECTIVES

M.K. McAllister, E.K. Pikitch, and E.A. Babcock 1871–1890 Using demographic methods to construct Bayesian priors for the intrinsic rate of increase in the Schaefer model and implications for stock rebuilding

Larry D. Jacobson, Jose A.A. De Oliveira, Manuel Barange, Miguel A. Cisneros-Mata, Roberto Félix-Uraga, John R. Hunter, Jin Yeong Kim, Yasunobu Matsuura, Miguel Ñiquen, Carmela Porteiro, Brian Rothschild, Ramiro P. Sanchez, Rodolfo Serra, Andres Uriarte, and Tokio Wada 1891–1903 Surplus production, variability, and climate change in the great sardine and anchovy fisheries

National Research Council Canada
Ottawa, Canada
K1A 0R6

Conseil national de recherches Canada
Ottawa, Canada
K1A 0R6

Postage paid at Ottawa
Publications mail
Registration No. 1240579

Port payé à Ottawa
Poste-publication
Enregistrement n° 1240579

Canadian Journal of Fisheries and Aquatic Sciences

Volume 58, Number 9, September 2001

Journal canadien des sciences halieutiques et aquatiques

Volume 58, numéro 9, septembre 2001

RAPID COMMUNICATION / COMMUNICATION RAPIDE

Kenneth T. Frank and Nancy L. Shackell **1703–1707** Area-dependent patterns of finfish diversity in a large marine ecosystem

ARTICLES / ARTICLES

S. Ballance, P.J. Phillips, C.R. McCrohan, J.J Powell, R. Jugdaohsingh, and K.N. White **1708–1715** Influence of sediment biofilm on the behaviour of aluminum and its bioavailability to the snail *Lymnaea stagnalis* in neutral freshwater

Robert J. Latour, John M. Hoenig, John E. Olney, and Kenneth H. Pollock **1716–1726** Diagnostics for multiyear tagging models with application to Atlantic striped bass (*Morone saxatilis*)

Marianne E. Meding and Leland J. Jackson **1727–1736** Biological implications of empirical models of winter oxygen depletion

Marie-Noële Croteau, Landis Hare, and André Tessier **1737–1746** Differences in Cd accumulation among species of the lake-dwelling biomonitor *Chaoborus*

Florian Mermillod-Blondin, Magali Gérino, Valérie Degrange, Robert Lensi, Jean-Luc Chassé, Michael Rard, and Michel Creuzé des Châtelliers **1747–1759** Testing the functional redundancy of *Limnodrilus* and *Tubifex* (Oligochaeta, Tubificidae) in hyporheic sediments: an experimental study in microcosms

Shelton J. Harley, Ransom A. Myers, and Alistair Dunn **1760–1772** Is catch-per-unit-effort proportional to abundance?

Stephen C. Schroeter, Daniel C. Reed, David J. Kushner, James A. Estes, and David S. Ono **1773–1781** The use of marine reserves in evaluating the dive fishery for the warty sea cucumber (*Parastichopus parvimensis*) in California, U.S.A.

Yong Cao, David P. Larsen, and Robert M. Hughes **1782–1793** Evaluating sampling sufficiency in fish assemblage surveys: a similarity-based approach

Jason E. Tanner **1794–1804** Influence of harvest refugia on penaeid prawn population dynamics and sustainable catch

Kentaro Morita and Takashi Matsuishi **1805–1811** A new model of growth back-calculation incorporating age effect based on otoliths

Continued on inside back cover / Suite au verso

Canada

Printed in Canada by / Imprimé au Canada par
INTRA MEDIA INC./datamark systems
Typesetting by / Typographie par
NRC Research Press / Presses scientifiques du CNRC

0706-652X(200109)58.9;1-X